



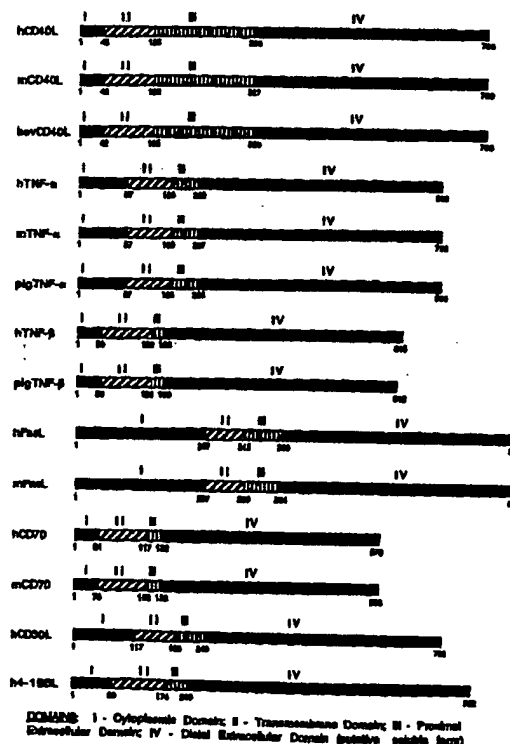
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(54) Title: NOVEL EXPRESSION VECTORS CONTAINING ACCESSORY MOLECULE LIGAND GENES AND THEIR USE FOR IMMUNOMODULATION AND TREATMENT OF MALIGNANCIES AND AUTOIMMUNE DISEASE

(57) Abstract

This invention relates to genes which encode accessory molecule ligands and their use for immunomodulation, vaccination and treatments of various human diseases, including malignancies and autoimmune diseases. This invention also describes the use of accessory molecule ligands which are made up of various domains and subdomain portions of molecules derived from the tumor necrosis factor family. The chimeric molecules of this invention contain unique properties which lead to the stabilization of their activities and thus greater usefulness in the treatment of diseases. Vectors for expressing genes which encode the molecules of this invention are also discussed.



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DESCRIPTIONNovel Expression Vectors Containing Accessory Molecule
Ligand Genes And Their Use For Immunomodulation And
Treatment Of Malignancies And Autoimmune DiseaseRelated Application

This application claims priority to Kipps et al.,
NOVEL EXPRESSION VECTORS CONTAINING ACCESSORY MOLECULE
LIGAND GENES AND THEIR USE FOR IMMUNOMODULATION AND
5 TREATMENT OF MALIGNANCIES, United States Provisional
Application No. 60/132145, filed December 9, 1996, which
is incorporated herein by reference including drawings.

Technical Field of the Invention

The present invention relates to novel expression
10 vectors containing genes which encode an accessory
molecule ligand and the use of those vectors for
immunomodulation, improved vaccination protocols and the
treatment of malignancies and autoimmune diseases. More
particularly, this invention provides expression vectors
15 and methods for treating various neoplastic or malignant
cells, and expression vectors and methods for treating
autoimmune Disease. This invention also contemplates
the production and expression of accessory molecule
ligands with greater stability and enhanced function.

20 Background of the Invention

Leukemias, lymphomas, carcinomas and other
malignancies are well known and described in, e.g.,
Harrison's Principles of Internal Medicine, Wilson et
al., eds., McGraw-Hill, New York, pp. 1599-1612. These
25 malignancies appear to have somehow escaped the immune
system surveillance mechanisms that eliminate rapidly
and continuously proliferating cells. The exact
mechanism by which these malignancies escape the immune
system surveillance is not known.

Some of these malignant immune system cells are malignant antigen presenting cells which do not function properly within the immune cascade. For example, neoplastic B cells cannot induce even weak allogeneic or autologous mixed lymphocyte reactions in vitro. Further evidence that malignancies survive due to the failure of the immune surveillance mechanism includes the increased frequency of such malignancies in immunocompromised individuals, such as allograft recipients and those receiving long-term immunosuppressant therapy. Further, the frequency of these malignancies is increased in patients having Acquired Immune Deficiency Syndrome (AIDS) and patients with primary immune deficiency syndromes, such as X-linked lymphoproliferative syndrome or Wiscott-Aldrich Syndrome (Thomas et al., Adv. Cancer Res. 57:329, 1991).

The immune system normally functions to eliminate malignant cells by recognizing the malignant cells as foreign cells and clearing those cells from the body. An immune reaction depends on both the immune system's antibody response and on the cellular immune response within a patient. More specifically, the cellular immune response which acts to recognize the malignant cells as foreign requires a number of different cells of the immune system and the interaction between those cells. An immune reaction begins with a T lymphocyte (T cell) which has on its cell surface the T cell receptor. The T cell also has the ability to express on its surface various accessory molecules which interact with accessory molecules on the B lymphocyte (B cell). When the T cell receptor of the T cell specifically binds to a foreign antigen, such as a malignant cell, it becomes activated and expresses the accessory molecule ligand, CD40 ligand on its cell surface. The accessory cell molecule ligand is only present on the activated T cells for a short period of time and is rapidly removed from the cell surface. After the accessory cells molecule

ligand is removed from the surface of the activated T cell, its ability to bind to B cells via the accessory molecule ligand is destroyed.

When present on the surface of an activated T cell,
5 the accessory cell ligand can specifically bind to the accessory cell molecule present on the B cell. This specific T-B cell interaction causes the B and T cell to express costimulatory surface accessory molecule and cytokines which result in an immune activation which
10 lead to cytolytic T cells which specifically kill and remove the malignant cell from the body.

The interaction with an activated T cell is not solely limited to B cells but rather can be carried out by any cell which is able to present antigen to the T
15 cell (an antigen presenting cell). These cells include B lymphocyte, macrophages, dendritic cells, monocytes, Langerhans cells, interdigitating cells, follicular dendritic cells or Kupffer cells. These cells all are known to have various accessory molecules on the cell
20 surface which allow them to interact with other cells of the immune system. For example, these antigen presenting cells all have the accessory molecule CD40 on their cell surface. The presence of these accessory molecules allows these antigen presenting cells to
25 specifically bind to complimentary accessory molecule ligand and thus directly interact with other immune cells.

A large number of accessory molecule ligands are members of the tumor necrosis factor superfamily.
30 (Fanslow et al., Sem. Immun., 6:267-268 (1994)). The genes for a number of these accessory molecule ligands have been cloned and identified. These accessory molecule ligand genes encode accessory molecules which all have the configuration of Type II membrane proteins
35 and exhibit varying degrees of homology with other accessory molecule ligand genes. For example, the accessory molecule ligand genes encoding both murine

CD40 ligand and human CD40 ligand have been isolated. See, Armitage et al., Nature, 357:80-82 (1992) and Hollenbaugh et al., EMBO J., 11:4313-4321 (1992).

CD40 and its ligand, CD40 ligand are critical
5 components of a normal immune response. CD40 mediated signals induce immune lymphocytes to proliferate and differentiate and become potent antigen presenting cells. Malignant or neoplastic B cells are poor antigen
10 presenter cells and are unable to stimulate a vigorous allogeneic mixed lymphocyte reaction. Successful cross linking of CD40 molecules on immune cells results in a strong allogeneic mixed lymphocyte reaction suggesting a strong immune reaction. Various soluble CD40 ligands or antibodies specific for CD40 have been used to
15 potentially cross link CD40. These soluble CD40 ligands and CD40-specific antibodies are not optimal for cross linking the CD40 molecules on antigen presenting cells and do not work as effectively as CD40 ligand expressed on a cell membrane to produce strong stimulation of
20 antigen presenting cells. These methods are also difficult to implement because large amounts of CD40 ligand constructs or antibodies must be isolated which is difficult and time-consuming work. Other strategies to utilize CD40 ligand in solution or as a membrane
25 bound molecule including transformation of fibroblasts with CD40 ligand to produce cultured cells which are then used to present antigen are not amenable to in vivo human clinical protocols.

CD95 (Fas) interaction with its ligand (Fas-ligand,
30 or FasL) functions to limit the duration of the immune response and/or life-span of activated lymphocytes. Apoptosis induced by Fas-FasL binding serves to clear activated self-reactive lymphocytes. Problems caused by altering this pathway have been demonstrated in animals
35 with defects in Fas<->Fas-ligand interactions. Mice having mutations, which inactivate CD95 or FasL, develop numerous disorders including autoimmune pathology

resembling that seen in patients with rheumatoid arthritis (RA) or systemic lupus. Zhang, et al., in J. Clin. Invest. 100:1951-1957 (1997) show that injection of FasL-expressing virus, into the joints of mice with collagen-induced-arthritis, results in apoptosis of synovial cells and relief of arthritis symptoms. Expression of Fas ligand allows clearance of activated cells which play a role in the pathogenesis of autoimmune disease. Therefore, a gene therapy strategy for introducing FasL into the joints of rheumatoid arthritis patients could function to improve disease pathology by leading to destruction of the infiltrating mononuclear cells.

Administration of soluble accessory molecules and accessory molecule ligands has been shown to trigger or to be associated with adverse physiological effects. For example, treatment of mice, having wild-type CD40-receptor expression, with soluble CD40L-CD8 fusion protein resulted in a pulmonary inflammatory response. This was not observed in mice in which the gene for the CD40 receptor had been knocked out. These experiments, described in Wiley, J.A. et al., Journal of Immunology 158:2932-2938 (1997), support in vitro data which suggest that CD40 ligation can result in inflammatory responses.

Direct administration of purified recombinant soluble Tumor Necrosis Factor (either α or β) results in shock and tissue injury, as described in Tracey, K. J., and A. Cerami, Annu. Rev. Med. 45:491-503 (1994). Within minutes after acute intravenous or intra-arterial administration of TNF, a syndrome of shock, tissue injury, capillary leakage syndrome, hypoxia, pulmonary edema, and multiple organ failure associated with a high mortality ensues. Chronic low dose of TNF causes anorexia, weight loss, dehydration and depletion of whole-body protein and lipid.

Soluble Fas ligand and receptor have also been shown to be associated with tissue damage and other adverse effects. CD95, the Fas receptor, is a mediator of apoptosis. Fas ligand induces apoptosis by binding to Fas receptor. As shown in Galle, P.R., et al., J. Exp. Med. 182:1223-1230 (1995) administering an agonistic anti-Fas antibody resulted in liver damage to mice. Mice injected intraperitoneally with the agonistic antibody died within several hours, and analyses revealed that severe liver damage by apoptosis was the most likely cause of death.

The role of soluble Fas ligand (FasL), in the pathogenesis of systemic tissue injury in aggressive lymphoma is described in Sato, K. et al., British Journal of Haematology, 94:379-382 (1996). The findings presented in this report indicate that soluble FasL is directly associated with the pathogenesis of liver injury and pancytopenia.

CD27, the receptor for the accessory molecule ligand, CD70, was shown, in a report written by van Oers, et al., in Blood 82:3430-3436 (1993), to be associated with B cell malignancies.

The above findings all contraindicate the administration of soluble accessory molecule ligands, highlighting the need for therapies that increase the levels of these molecules without resulting in an elevation of their soluble forms.

Despite the wealth of information regarding accessory molecule ligand genes and their expression on the surface of various immune cells, the exact mechanism by which the accessory molecule ligand genes are regulated on antigen presenting cells is not yet known. Without specific knowledge of the regulation of expression of accessory molecule ligand genes on these antigen presenting cells, altering the immune response by varying expression of an accessory molecule ligand gene has to date not been possible. Without any

specific knowledge as to how to regulate the expression of an accessory molecule ligand gene on an antigen presenting cell, it is not possible to alter the immune response towards malignant cells. Thus, there was a
5 need for a method of increasing the expression of an accessory molecule ligand gene on normal and malignant cells including antigen presenting cells.

Further, without the ability to regulate the expression of accessory molecule ligands, it is not
10 possible to alter the immune clearance of these cells.

Summary of the Invention

The present invention fills these needs by providing novel expression vectors containing accessory molecule ligand genes and methods for introducing those
15 genes into normal and malignant antigen presenting cells thereby allowing the alteration of an immune response, the treatment of autoimmune diseases and the treatment of various neoplasias. This invention provides vectors, including gene therapy vectors which contain accessory
20 molecule ligand genes. These vectors also contain the additional genetic elements, such as promoters, enhancers, polyadenylation signals (3' ends), which allow that vector to be successfully placed within the cell and to direct the expression of the accessory
25 molecule ligand gene in a cell. Such gene therapy vectors are capable of transforming animal cells directly and thereby introducing the accessory molecule ligand gene into the cells of that animal in a form which can be utilized to produce accessory molecule
30 ligands within that cell.

In other aspects of the present invention, the function of an accessory molecule ligand is modified by altering the half life of the molecule on the cell surface or by changing the level of expression of that
35 molecule on the cell surface. In preferred embodiments, the present invention provides accessory molecule

ligands which are modified to improve the stability of such accessory molecule ligands on the cell surface. Such increased stability may be accomplished using any of the disclosed methods of molecules described in this application, including chimeric molecules and molecules into which mutations have been introduced at least one location. The present invention also contemplates increasing the expression of such a molecule.

The present invention also provides gene therapy vectors containing the accessory molecule ligand genes which are chimeric in that portions of the gene are derived from two separate accessory molecule ligands which may or may not be from different species. The accessory molecule ligand genes of the present invention include genes which encode molecules of the tumor necrosis factor (TNF) family. The molecules which make up the TNF family include TNF α , TNF β , CD40 ligand, Fas ligand, CD70, CD30 ligand, 41BB ligand (4-1BBL), nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL). In some embodiments of the present invention, the chimeric accessory molecule ligand genes of the present invention contain at least a portion of a murine accessory molecule ligand gene together with portions of accessory molecule ligand genes derived from either mouse, humans or other species. Some preferred embodiments of the present invention utilize murine CD40 ligand genes and chimeric CD40 ligand genes containing at least a segment of the murine CD40 ligand gene together with at least a segment of the human CD40 ligand gene. The present invention contemplates chimeric accessory molecule ligand genes wherein segments from the accessory molecule ligand gene of one species have been interchanged with segments from a second accessory molecule ligand gene which may optionally be from a different species. For example, in one preferred embodiment, the murine CD40 ligand gene

transmembrane and cytoplasmic domains have been attached to the extracellular domains of human CD40 ligand gene.

The present invention contemplates gene therapy vectors which are capable of directly infecting the
5 human, mammal, insect, or other cell. The use of such gene therapy vectors greatly simplifies inserting an accessory molecule ligand gene into those cells. The contemplated gene therapy vectors may be used in vivo or in vitro to infect the desired cell and are particularly
10 useful for infecting malignant cells to effect sustained high-level expression of a physiologic ligand.

The present invention also contemplates animal, mammal, and human cells containing a gene therapy vector which includes an accessory molecule ligand gene and
15 sufficient genetic information to express that accessory molecule ligand within that cell. In preferred embodiments, the present invention also contemplates human neoplastic antigen presenting cells which contain the gene therapy vectors of the present invention or
20 contain an accessory molecule ligand gene together with a promoter and 3' end region.

The present invention also contemplates human cells and human neoplastic cells containing a gene therapy vector which includes a chimeric accessory molecule
25 ligand gene. The present invention also contemplates bacterial cells or animal cells containing accessory molecule ligand genes, chimeric accessory molecule ligand genes, murine accessory molecule ligand genes, human accessory molecule ligand genes, the gene therapy
30 vectors of the present invention, the vectors of the present invention, and a chimeric accessory molecule ligand gene together with a heterologous promoter, enhancer or polyadenylation sequence.

The present invention also contemplates methods of
35 altering immune response within a human patient or the immunoreactivity of human cells in vivo by introducing a gene which encodes an accessory molecule ligand gene

into the human cells so that that accessory molecule ligand is expressed on the surface of those human cells. This method includes the introduction of the accessory molecule ligand gene as part of a gene therapy vector or
5 in association with a heterologous or native promoter, enhancer or polyadenylation signal. Some preferred embodiments of the present invention utilize introduction of Fas ligand genes and chimeric Fas ligand genes, constructed as contemplated above for CD40, into
10 human cells to alter their immunoreactivity. The present invention also includes methods in which such accessory molecule ligand genes are inserted into cells which have the accessory molecule to which the accessory molecule ligand binds on the surface of the cell into
15 which the accessory molecule ligand gene.

The present methods of altering immunoreactivity are applicable to all types of human, animal, and murine cells including human neoplastic cells such as human lymphomas, leukemias and other malignancies. In
20 preferred embodiments, this method is used to introduce the gene encoding the accessory molecule ligand into potential antigen presenting cells of a human patient or cell which can stimulate bystander antigen presenting cells. Such antigen presenting cells include monocytes,
25 macrophages, B cells, Langerhans cells, interdigitating cells, follicular dendritic cells, Kupffer cells, and the like. The various antigen presenting cells may be present as part of a known malignancy in a human patient such as leukemias, lymphomas, acute monocytic leukemia
30 (AML), chronic lymphocytic leukemia (CLL), acute myelomonocytic leukemia (AMML), chronic myelogenous or chronic myelomonocytic leukemia (CMML) and thus would include all tumors of any cell capable of presenting antigen to the human or animal immune system or are
35 capable of stimulating bystander antigen presenting cells. The present invention also contemplates modulating the immune system by introducing genes

encoding an accessory molecule ligand gene of the present invention into any number of different cells found in a patient, including muscle cells, skin cells, stromal cells, connective tissue cells, fibroblasts and
5 the like.

The present invention also contemplates methods of treating neoplasias in either a human patient or an animal patient. In one preferred embodiment, the method comprises isolating the neoplastic cells from the human
10 or animal patient and inserting into those isolated cells the gene which encodes the chimeric accessory molecule ligand or the accessory molecule ligand so that that molecule is expressed on the cell surface of those neoplastic cells or other somatic cells. The neoplastic
15 cells are then infused back into the human or animal patient and may then participate in an enhanced immune response.

The present invention also contemplates the co-infection or co-introduction of the accessory molecule
20 ligand gene together with a gene which encodes a tumor or carcinoma specific antigen. This combination of molecules are then expressed on the surface of the neoplastic cells and when those cells are introduced into the patient lead to the rapid immune response
25 resulting in the destruction of those cells.

The present methods also include directly introducing the gene therapy vector or other vector carrying the accessory molecule ligand gene directly into the tumor or tumor bed of a patient. Upon entering
30 the tumor bed of the patient, the gene therapy vector or other vector enter the cells present in the tumor or tumor bed and then express the accessory molecule ligand gene on the surface of those cells. These cells then are able to participate fully in the human immune or
35 animal immune response.

The present invention also contemplates methods of augmenting an immune response to a vaccine. The present

method of vaccinating an animal against a predetermined organism or antigen by administering to that animal a vaccine which has a genetic vector containing an accessory molecule ligand gene. Other embodiments of the present invention include vaccinating an animal by administering two separate genetic vectors, one containing the antigens from the organism to which immunity is desired by isolating the cells of the target animal and contacting with those cells a vector encoding at least one antigen from a predetermined organism so that the antigen is expressed by the cells and also contacting those cells with a different vector which expresses the accessory molecule ligand gene on the surface of the animal's antigen presenting cells. Together these two separate vectors produce a vaccination which is much stronger and of longer duration than is vaccination with antigen alone.

The present methods of vaccination are applicable to vaccinations designed to produce immunity against a virus, a cell, a bacteria, any protein or a fungus. The present methods are also applicable to immunization against various carcinomas and neoplasias. In these embodiments, the tumor antigen against which immunity is desired is introduced into the animal together with the genetic vector containing the accessory molecule ligand gene.

The present invention also contemplates methods of treating arthritis utilizing a gene therapy vector encoding an accessory molecule ligand. Of particular interest for use with arthritis is the Fas ligand molecule in which the expression of Fas ligand activity has been increased in the joint and/or the stability of the Fas ligand activity on cells within the joint enhanced. In other embodiments, the present invention contemplated methods of treating arthritis utilizing chimeric accessory molecule ligands and chimeric accessory molecule ligand genes. The present invention

also contemplates both ex vivo therapy and in vivo therapy of arthritis utilizing the expression vectors of the present invention together with the Fas ligand and modified versions of that molecule including chimeric
5 molecules.

Brief Description of the Drawings

Figure 1. Figure 1 is a diagram showing a number of accessory molecule ligand genes and Domains I-IV of those genes as deduced from sequence data.

10 Figure 2. Figure 2 is a diagram showing example chimeric accessory molecule ligand genes. The domains derived from the murine accessory module are shown shaded.

Figure 3. Figure 3 shows the amount of either
15 mouse or human CD40 ligand found on the surface of Hela or CLL cells infected with gene therapy vectors containing the genes encoding these molecules. Figure 3A shows uninfected Hela cells (shaded) and Hela cells infected with a gene therapy vector encoding murine CD40
20 ligand. Figure 3B shows uninfected Hela cells (shaded) and Hela cells infected with a gene therapy vector encoding human CD40 ligand. Figure 3C shows uninfected CLL cells (shaded) and CLL cells infected with a gene therapy vector encoding murine CD40 ligand. Figure 3D
25 shows uninfected CLL cells (shaded) and CLL cells infected with a gene therapy vector encoding human CD40 ligand.

Figure 4. Figure 4 shows histograms of the increased expression of CD54 (Figure 4B) and CD80
30 (Figure 4D) on CLL cells into which a gene therapy vector containing the accessory molecule ligand gene (murine CD40 ligand gene) has been introduced. The shaded graph indicates control stain in FACS analysis and the open graph indicates staining with monoclonal
35 antibodies immunospecific for either CD54 (Figures 4A and 4B) or CD80 (Figures 4C and 4D).

Figure 5. Figure 5 shows the cell proliferation as measured by ^3H -TdR incorporation of allogeneic T cells in response to various stimulation regimes. The CLL cells containing a gene therapy vector expressing an accessory molecule ligand gene (the murine CD40 ligand gene) were introduced, stimulating allogeneic T cells to proliferate.

Figure 6. Figure 6 shows the production of gamma interferon (IFNg) by allogeneic T cells stimulated with CLL cells containing an accessory molecule ligand gene.

Figure 7. Figure 7 shows the treatment of a neoplasia in an animal using a gene therapy vector containing an accessory molecule ligand gene of the present invention. The open squares show mice immunized with neoplastic cell not expressing an accessory molecule ligand of the present invention. Mice immunized with neoplastic cells expressing an accessory molecule ligand of the present invention are shown as the horizontal line at the top of the Figure and show no morbidity.

Figure 8. Figure 8 shows the production levels and stabilities of CD40 ligand and CD40 ligand transcript in CLL (upper graph) and normal blood mononuclear cells (lower graph).

Figure 9. Figure 9 shows the time course of transgene expression in CLL B cells infected with the accessory molecule ligand (CD40 ligand). The MFIR (mean fluorescence intensity ratio), comparing the fluorescence intensity of CD19⁺ CLL cells stained with PE-labeled CD40 ligand versus the same stained with a PE-labeled isotype control mAb at each time point, are represented by the closed circles connected by solid lines according to the scale provided on the left-hand ordinate.

Figure 10. Figure 10 shows changes in surface antigen phenotype of CLL B cells infected with a gene therapy vector containing an accessory molecule ligand,

CD40 ligand. Shaded histograms represent staining of uninfected CLL cells (thin lines) stained with nonspecific control antibody, open histograms drawn with thin lines represent uninfected CLL cells stained with FITC-conjugated specific mAb, and open histograms drawn with thick lines (labeled CD154-CLL) represent CLL cells infected with the accessory molecule ligand gene therapy vector and stained with FITC-conjugated specific mAb.

Figure 11. Figure 11 shows levels of CD27 produced in CLL cells infected with a gene therapy vector containing an accessory molecule ligand. Figure 11A shows that CD40L-infected CLL (CD154-CLL) cells express reduced levels of surface CD27. Open histograms represent staining of non-infected CLL cells (thin lines) or infected CLL (thick lines) with FITC-conjugated aCD27 mAb, respectively. Figure 11B shows production of soluble form of CD27 by CLL B cells.

Figure 12. Figure 12 shows allogeneic T cell responses induced by CLL cells infected with a gene therapy vector containing an accessory molecule ligand (CD40 ligand, also called CD154). Figure 12A indicates the concentration of IFN γ in the supernatants after stimulation of allogeneic T cells with CLL cells containing the accessory molecule ligand. Figure 12B shows cell proliferation, as assessed by incorporation of ³H-thymidine. Figures 12C and 12D show secondary allogeneic T cell responses induced by CLL containing the accessory molecule ligand.

Figure 13. Figure 13 depicts autologous T cell responses induced by CLL B cells containing the accessory molecule ligand, CD40 ligand or CD154, and controls. Figure 13A shows incorporation of ³H-thymidine by autologous T cells co-cultured with the CLL cells. Figure 13B shows the levels of human IFN γ produced by autologous T cells co-cultured with the CLL cells. In Figure 13C, the CTL activities of autologous T cells

induced by CLL B cells containing the accessory molecule ligand are graphed.

Figure 14. Figure 14 shows specificity of CTL for autologous CLL B cells. IFN γ concentration was assessed in the supernatants after 48 h of culture (Figure 14A), and cytolytic activity was assessed at 3 h of culture (Figure 14B). In Figure 14C, mAb were added to the autologous leukemia target cells prior to the CTL assay.

Figure 15. Figure 15 shows that intercellular stimulation plays a role in production of the phenotypic changes observed in CLL cells expressing the accessory molecule ligand. In Figure 15A, the effect of culture density on the induced expression of CD54 and CD80 following infection with a gene therapy vector containing the accessory molecule ligand (CD40 ligand, CD154) is shown. Shaded histograms represent staining of leukemia B cells with a FITC-conjugated isotype control mAb. Open histograms represent CD154-CLL B cells, cultured at high or low density (indicated by arrows), and stained with a FITC-conjugated mAb specific for CD54 or CD80. Figure 15B shows inhibition of CD154-CLL cell activation by anti-CD154 mAb. Figures 15C and 15D depict expression of immune accessory molecules on bystander non-infected CLL B cells induced by CLL cells expressing the accessory molecule ligand. Shaded histograms represent staining with PE-conjugated isotype control mAb.

Figure 16. Figure 16 shows that the vector encoding an accessory molecule ligand enhances immunization against β -gal in mice. Figure 16A shows that mice that received intramuscular injections of the pCD40L vector produced significantly more antibodies to β -gal than did mice injected with either the non-modified pcDNA3 vector or pCD40L. Figure 16B, ELISA analyses of serial dilutions of sera collected at d28, shows that mice co-injected with placZ and pCD40L had an

eight-fold higher mean titer of anti- β -gal antibodies at d28 than mice treated with placZ + pCDNA3.

Figure 17. Figure 17 shows analysis of the IgG₁ and IgG_{2a} immune responses to intramuscular plasmid DNA immunizations with and without a vector, pCD40L, encoding an accessory molecule ligand. IgG_{2a} anti- β -gal antibodies predominated over IgG₁ subclass antibodies in the sera of mice injected with either placZ and pCDNA3 or placZ and pCD40L. In contrast, BALB/c mice injected with β -gal protein developed predominantly IgG₁ anti- β -gal antibodies, and no detectable IgG_{2a} anti- β -gal antibodies.

Figure 18. Figure 18 shows the comparison between injection of mice with a vector, pCD40L, encoding an accessory molecule ligand, at the same and different sites as placZ. Adjuvant effect of pCD40L requires co-injection with placZ at the same site.

Figure 19. Figure 19 shows that co-injection into dermis of a vector encoding an accessory molecule ligand, pCD40L, with placZ enhances the IgG anti- β -gal response in BALB/c mice.

Figure 20. Figure 20 shows that a vector encoding an accessory molecule ligand, pCD40L, enhances the ability of placZ to induce CTL specific for syngeneic β -gal-expressing target cells. Splenocyte effector cells, taken from mice which had received injections of placZ and pCD40L, specifically lysed significantly more cells than did splenocytes from mice that received control injections.

Figure 21. Figure 21 shows downmodulation of human CD40L, but not murine CD40L, in lung tumor cell lines that express CD40.

Figure 22. Figure 22A shows that CD40 binding induces enhanced expression of the tumor cell surface markers CD95 (Fas), CD54 (ICAM-1), and MHC-I, in lung tumor cell lines. Figure 22B shows downmodulation of human CD40L by CD40-positive tumor cells.

Figure 23. Figure 23 shows the inhibition of Fas ligand expression by lymphocytes in the presence of RA synovial fluid.

Figure 24. Figure 24 shows an outline for a
5 clinical trial of an accessory molecule ligand (CD40L) gene therapy treatment for B cell CLL.

Figure 25. Figure 25 shows a sequence line-up of human Fas ligand with human Fas ligand in which Domain III is replaced by Domain III of murine Fas ligand. The
10 top protein sequence is native human Fas ligand. Domain III is underlined with the dotted line. The double underline indicates a putative MMP cleavage site. The bottom protein sequence is that of chimeric human-mouse Fas ligand. Domain III of the mouse Fas ligand
15 (underlined with dotted line) is substituted for Domain III of human Fas ligand. The numbers correspond to the amino acid sequence number using 1 for the start of the polypeptide sequence. The number of the first nucleotide base for the codon encoding the amino acid is
20 $1+3x(n-1)$, where n is the amino acid sequence number.

Figure 26. Figure 26 shows a sequence line-up of human Fas ligand with human Fas ligand in which Domain III has been replaced with Domain III of human CD70. The top protein sequence is native human Fas ligand, and
25 the bottom sequence is that of chimeric Fas ligand, in which Domain III of human CD70 has been substituted for Fas Domain III. Other markings are used similarly as in Figure 25.

Figure 27. Figure 27 shows a sequence line-up of
30 human Fas ligand with human Fas ligand in which Domain I has been replaced with Domain III of human CD70. The top protein is native human Fas ligand, and the bottom protein sequence is that of chimeric Fas ligand, in which Domain III has been replaced with Domain I of
35 human CD70. Other markings are used similarly as in Figure 25.

Figure 28. Figure 28 shows the amino acids around and at known matrix metalloproteinase (MMP) cleavage sites, as described in Smith, M.M. et al., Journal of Biol. Chem. 270:6440-6449 (95) and Nagase, H., and G.B. Fields, Biopolymers (Peptide Science) 40:399-416 (96). The cleavage site is indicated with an arrow.

Detailed Description of the Invention

All references cited herein are hereby incorporated in their entirety by reference.

10 I. Definitions

An "accessory molecule ligand gene" is a gene which encodes all or part of an accessory molecule ligand. The gene comprises at least the nucleotide sequence required to encode the functional portion of an accessory molecule ligand. The gene may optionally include such genetic elements as promoters, enhancers and 3' ends. The accessory molecule ligand gene is derived from a ligand which is a member of the tumor necrosis factor (TNF) family, including CD40 ligand, Fas ligand, CD70, TNF α , TNF β , CD30 ligand, 4-1BB ligand (4-1BBL), nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL). As used herein, the term "accessory molecule ligand gene" includes chimeric accessory molecule ligand genes as defined below.

25 As used herein, the term "malignant cells or neoplastic cells," is defined to mean malignant or cancerous cells which are found in a human patient or an animal. Preferred types of malignant or neoplastic cells include any malignant antigen-presenting cell. In some preferred embodiments, these malignant antigen presenting cells have at least low levels of CD40 present on the cell surface.

As used herein, the term "neoplastic human cells" is defined to mean human cells which are neoplastic including but not limited to antigen presenting cells,

any neoplastic cell which may function as an antigen presenting cell or function to facilitate antigen presentation, neoplastic monocytes, neoplastic macrophages, neoplastic B cells, neoplastic dendritic cells, neoplastic Langerhans cells, neoplastic interdigitating cells, neoplastic follicular dendritic cells, or neoplastic Kupffer cells and the like. The definition of neoplastic human cells includes those cells which are associated with neoplastic cells in the tumor bed of human patients. Typically, the neoplastic human cells are either leukemias, lymphomas, AML, ALL, AMML, CML, CMML, CLL other tumors of antigen presenting cells or breast, ovarian or lung neoplastic cells. It is also contemplated that the accessory molecule ligand genes or chimeric accessory molecule ligand genes of the present invention may be inserted into somatic cells. These somatic cells can be created by a genetic engineering process which has introduced into those cells genes which encode molecules which render those cells capable of presenting antigen to the immune system.

As used herein, the term "chimeric gene" is defined to mean a gene in which part of the gene is derived from a second different gene and combined with the first gene so that at least a portion of each gene is present in the resulting chimeric gene. A gene may be chimeric if any portion of the sequence which encodes the resulting protein is derived from a second and different gene. Typical chimeric genes include genes in which specific functional domains from one gene have been transferred to a second gene and replace the analogous domains of that second gene. For example, the resulting chimeric gene may have one domain derived from a murine gene and several domains derived from a human gene. These domains may range in size from 5 amino acids to several hundred amino acids. Other examples of chimeric accessory molecule ligand genes include genes which

contain nucleotides encoding amino acids not found in any naturally occurring accessory molecule ligand gene. Examples of chimeric genes and potential various combinations of domains are numerous and one of skill in the art will understand that no limit is placed on the amount of one gene that must be present in a second gene to render it chimeric.

As used herein, the term "murine CD40 ligand gene" is defined to mean an accessory molecule ligand gene which is derived from a murine CD40 ligand gene. Examples of such murine CD40 ligand genes include the gene isolated by Armitage et al., Nature, 357:80-82 (1992) and other genes derived from murine origin which hybridize to the gene described by Armitage et al. under low stringency hybridization conditions.

As used herein, the term "vector or genetic vector" is defined to mean a nucleic acid which is capable of replicating itself within an organism such as a bacterium or animal cell. Typical genetic vectors include the plasmids commonly used in recombinant DNA technology and various viruses capable of replicating within bacterial or animal cells. Preferred types of genetic vectors includes plasmids, phages, viruses, retroviruses, and the like.

As used herein, the term "gene therapy vector" is defined to mean a genetic vector which is capable of directly infecting cells within an animal, such as a human patient. A number of gene therapy vectors have been described in the literature, and include, the gene therapy vector described in Cantwell et al., Blood, In Press (1996) entitled "Adenovirus Vector Infection of Chronic Lymphocytic Leukemia B Cells." Such vectors have been described for example by Woll, P. J. and I. R. Hart, Ann. Oncol., 6 Suppl 1:73 (1995); Smith, K. T., A. J. Shepherd, J. E. Boyd, and G. M. Lees, Gene Ther., 3:190 (1996); Cooper, M. J., Semin. Oncol., 23:172 (1996); Shaughnessy, E., D. Lu, S. Chatterjee, and K. K.

Wong, Semin. Oncol., 23:159 (1996); Glorioso, J. C., N. A. DeLuca, and D. J. Fink, Annu. Rev. Microbiol., 49:675 (1995); Flotte, T. R. and B. J. Carter, Gene Ther., 2:357 (1995); Randrianarison-Jewtoukoff, V. and M. Perricaudet, Biologicals., 23:145 (1995); Kohn, D. B., Curr. Opin. Pediatr., 7:56 (1995); Vile, R. G. and S. J. Russell, Br. Med. Bull., 51:12 (1995); Russell, S. J., Semin. Cancer Biol., 5:437 (1994); and Ali, M., N. R. Lemoine, and C. J. Ring, Gene Ther., 1:367 (1994). All references cited herein are hereby incorporated by reference.

II. Genetic Vectors and Constructs Containing an Accessory Molecule Ligand Gene

A. Accessory Molecule Ligand Genes

In one embodiment of the present invention, preferred gene therapy vectors contain an accessory molecule ligand gene. This accessory molecule ligand gene may be derived from any source and may include molecules which are man-made and do not appear in nature. The present invention contemplates accessory molecule ligand genes which are derived from the genes encoding molecules within the tumor necrosis family (TNF) which includes the genes encoding: murine CD40 ligand, human CD40 ligand, Fas ligand, TNF α , TNF β , CD30 ligand, 4-1BB ligand, nerve growth factor, CD70, TNF-related apoptosis inducing ligand (TRAIL) and chimeric accessory molecule ligands. The nucleotide sequence of one accessory molecule ligand, the sequence of at least one form of the murine CD40 ligand gene, has been determined and is listed as SEQ ID NO: 1. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to the sequence present in SEQ ID NO: 1, and thus hybridizes to this sequence at low stringency hybridization conditions. One of skill in the art will understand that accessory molecule ligand genes, including murine CD40 ligand gene, useful

in the present invention may be isolated from various different murine strains.

The nucleotide sequence of a human CD40 ligand gene has been determined and is shown as SEQ ID NO: 2. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 2, and thus hybridizes to this sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the human CD40 ligand genes, useful in the present invention, may vary depending on the individual from which the gene is isolated and such variations may prove useful in producing unique accessory molecule ligand genes. The present invention contemplates the use of the domains, sub-domains, amino acid or nucleotide sequence of the human CD40 ligand and/or human CD40 ligand gene as part of a chimeric accessory molecule ligand or chimeric accessory molecule ligand gene.

The nucleotide sequence of a bovine CD40 ligand gene has been determined and is shown as SEQ ID NO: 8. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 8, and thus hybridizes to the sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the bovine CD40 ligand genes, may vary depending on the individual animal from which the gene is isolated and that such variations may prove useful in producing unique accessory molecule ligand genes.

The nucleotide sequence of human TNF α and human TNF β have been determined and are shown as SEQ ID NOS: 9 and 10, respectively. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to either human TNF α or human TNF β (SEQ ID NOS: 9 and 10, respectively), and thus hybridizes to these sequences at low stringency conditions. The

accessory molecule ligand genes useful in the present invention, including the human TNF_α and TNF_β genes, may vary depending on the particular individual from which the gene has been isolated and these variations may
5 prove useful in producing unique accessory molecule genes.

The nucleotide sequence of porcine TNF_α and TNF_β have been determined and are shown as SEQ ID NO: 11. The present invention contemplates the use of any
10 accessory molecule ligand gene which is homologous to either SEQ ID NO: 11, and thus would hybridize to these sequences at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the porcine TNF_α and
15 TNF_β genes, may vary depending on the particular animal from which the gene is isolated and that such variation may prove useful in producing unique accessory molecule genes.

The nucleotide sequence of a murine TNF_α gene has
20 been determined and is shown as SEQ ID NO: 12. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 12, and thus hybridizes to the sequence at low stringency conditions. One of ordinary skill in the art
25 will understand that the accessory molecule ligand genes, including the murine TNF_α gene may vary depending on the individual from which the gene is isolated and that these variations may prove useful in producing unique accessory molecule genes.

30 The nucleotide sequence of human Fas ligand and murine (C57BL/6) Fas ligand have been determined and are shown as SEQ ID NOS: 13 and 14, respectively. The nucleotide sequence of murine Balb/c Fas ligand is shown as SEQ ID NO: 31. The present invention contemplates
35 the use of any accessory molecule ligand gene which is homologous to any of SEQ ID NOS: 13, 14, and 31, and thus hybridizes to the sequences at low stringency

conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the human Fas ligand or murine Fas ligand genes may vary depending on the particular individual or animal from which the gene is isolated and that such variations may prove useful in producing any accessory molecule genes.

The nucleotide sequence of a human CD70 gene has been determined and is shown as SEQ ID NO: 15. The murine CD70 gene sequence has also been determined, and is shown as SEQ ID NO: 36 and was described by Tesselaar et. al, J. Immunol. 159:4959-65(1997). The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 15 or 36, and thus hybridizes to this sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the human CD70 gene may vary depending on the individual from which the gene is isolated and that these variations may prove useful in producing unique accessory molecule ligand genes.

The nucleotide sequence of human CD30 ligand gene has been determined and is shown as SEQ ID NO: 16. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 16, and thus hybridizes to this sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the human CD30 ligand gene, may vary depending on the individual from which the gene is isolated and that such variations may prove useful in producing unique accessory molecule ligand genes.

The present invention also contemplates variations and variants of the nucleotide sequences of the accessory molecule ligand genes provided herein which are caused by alternative splicing of the messenger RNA. This alternative splicing of the messenger RNA inserts

additional nucleotide sequences which may encode one or more optional amino acid segments which in turn allows the accessory molecule ligand encoded to have additional properties or functions.

5 The nucleotide sequence of a human and mouse 4-1BBL have been determined and are shown as SEQ ID NOS: 17 and 18, respectively. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to either SEQ ID NOS: 17 or 18, and thus
10 hybridizes to these sequences at low stringency conditions. One of ordinary skill in the art will understand that accessory molecule ligand genes, including the human 4-1BBL gene may vary depending on the individual from which it is isolated and that such
15 variations may prove useful in producing unique accessory molecule ligand genes.

 The present invention also contemplates chimeric accessory molecules containing any domain, sub-domain portion, or amino acid sequence encoded by the following
20 genes: bovine TNF- α (SEQ ID NO: 21), murine CD40 ligand (SEQ ID NO: 22), human nerve growth factor- β (SEQ ID NO: 23), murine nerve growth factor (SEQ ID NO: 24), rat Fas ligand (SEQ ID NO: 25), human TNF-related apoptosis inducing ligand (TRAIL) (SEQ ID NO: 41, Genbank
25 accession number U37518), murine TNF-related apoptosis inducing ligand (TRAIL) (SEQ ID NO: 42, Genbank accession number U37522), murine CD30-Ligand (SEQ ID NO: 43), human 4-1BBL (SEQ ID NO: 17), and murine 4-1BBL (SEQ ID NOS: 44 and 18). The present invention also
30 contemplates chimeric accessory molecules which utilize genes encoding amino acid sequences homologous to these sequences.

 The present invention contemplates chimeric accessory molecule ligand genes which are comprised of a
35 nucleotide segment derived from one accessory molecule ligand gene operatively linked to a nucleotide sequence

derived from a different accessory molecule ligand gene or other gene.

For example, chimeric accessory molecule ligand genes are contemplated which are comprised of a segment
5 of the murine CD40 ligand gene which has been operatively linked to at least one other additional gene segment derived from a different accessory molecule ligand gene. The size of the particular segment derived from the different accessory molecule ligand gene may
10 vary from a nucleotide sequence encoding a few amino acids, a sub-domain of the accessory molecule ligand, a domain of the accessory molecule ligand or more than a domain of an accessory molecule ligand. Other chimeric accessory molecules of the present invention are
15 comprised of an accessory molecule ligand gene into which nucleotides encoding an amino acid segment which is not found as part of a naturally occurring accessory molecule ligand have been inserted. This amino acid segment may be artificially created or derived from a
20 protein found in nature. The chimeric accessory molecule ligand gene encodes a chimeric amino acid sequence and thus a chimeric accessory molecule ligand encoded may possess unique properties in addition to the properties found on the individual segments derived from
25 the different accessory molecule ligand genes. The chimeric accessory molecule ligand gene may encode an accessory molecule ligand which has properties derived from the accessory molecule ligand used to construct the chimeric gene.

30 Each of the accessory molecule ligand genes which are a member of the tumor necrosis factor family have a similar secondary structure consisting of a number of domains. This domain structure includes a first domain which is encoded by the 5' region of the accessory
35 molecule ligand gene. The second domain (Domain II) is the domain which contains the amino acids which span the cell membrane and is thus called the transmembrane

domain. The third domain (Domain III) is the proximal extracellular domain and these amino acids are the amino acids which are found proximal to the cellular membrane. The fourth domain (Domain IV), is encoded by the 3' end
5 of the accessory molecule ligand gene and has been called the distal extracellular domain. The distal extracellular domain (Domain IV) generally makes up the soluble form of the tumor necrosis factor family molecule. Based on the x-ray crystal structure of human
10 TNF, the predicted secondary structure of the accessory molecule, CD40 ligand has been deduced together with the domain structure of these molecules by M. Peitsch and C. Jongeneel, International Immunology, 5:233-238 (1993). The secondary structures of the other members of the
15 tumor necrosis factor family were deduced using computer analysis together with comparison to the human TNF and CD40 ligand domain structure. In Table I, the domain boundaries of a number of accessory molecule ligand genes is shown. A diagram of these domains for a number
20 of these accessory cell molecule ligands is shown in Figure 1. The assignments of the domain boundaries are approximate and one of ordinary skill in the art will understand that these boundaries may vary and yet still provide useful identification of domains.

TABLE I
DOMAIN STRUCTURE OF TUMOR NECROSIS
FACTOR FAMILY MOLECULES*

		Domain I (Cytoplasmic)	Domain II (Transmembrane)	Domain III (Proximal Extracellular)	Domain IV (Distal Extracellular)
5	Human CD40 Ligand	1-42	43-135	136-330	331-786
	Murine CD40 Ligand	1-42	43-135	136-327	328-783
	Bovine CD40 Ligand	1-42	43-135	136-330	331-786
10	Human TNF- α	1-87	88-168	169-228	229-699
	Murine TNF- α	1-87	88-168	169-237	238-705
	Porcine TNF- α	1-87	88-168	169-228	229-696
	Human TNF- β	1-39	40-129	130-153	154-615
	Porcine TNF- β	1-39	40-126	127-150	151-612
	Human Fas Ligand	1-237	238-315	316-390	391-843
15	Murine Fas Ligand	1-237	238-309	310-384	385-837
	Human CD70	1-61	62-117	118-132	133-579
	Murine CD70	1-73	74-123	124-138	139-585
20	Human CD30 Ligand	1-117	118-186	187-240	241-702
	Murine CD30 Ligand	1-135	136-201	202-255	256-717
	Human 4-1BBL	1-69	70-174	175-210	211-762
25	Murine 4-1BBL	1-237	238-333	334-369	370-927
	Human TRAIL	1-39	40-117	118-375	376-843
	Murine TRAIL	1-51	52-111	112-387	388-873

30 * The Domains above are identified by the nucleotide boundaries of each domain using the first nucleotide of the initial methionine of the cDNA as nucleotide number 1.

One of ordinary skill in the art will understand that typical chimeric accessory molecule genes would include genes produced by exchanging domains or sub-domain segments between, for example, a mouse CD40 ligand gene and a human CD40 ligand gene. For example, chimeric accessory molecule gene may be constructed by operatively linking Domain I of the human CD40 ligand gene to Domains II-IV of the murine CD40 ligand gene. One of ordinary skill in the art will understand the variety of chimeric accessory molecule ligand genes which may be produced using the accessory molecules identified in Table I. The present invention also contemplates chimeric accessory molecules which are not shown in Table I but which are shown to have a similar domain structure. Other chimeric genes are also contemplated in which smaller segments (sub-domain segments) are exchanged between, for example, a murine CD40 ligand gene and a human CD40 ligand gene or a second murine CD40 ligand gene. One of skill in the art will understand that genes encoding accessory molecules will have at least gene segments which correspond to various functional segments of an accessory molecule ligand such as the murine CD40 ligand encoded by the murine CD40 ligand gene (SEQ ID NO: 1). It will also be apparent to one of skill in the art that the nucleotide boundaries identified in Table I may vary considerably from those identified for the murine CD40 ligand gene (SEQ ID NO: 1) and still define domains which are useful in the present invention.

In one preferred embodiment, the chimeric accessory molecule ligand gene is comprised of the nucleotides encoding extracellular domains (Domains III and IV) of human CD40 ligand operatively linked to the nucleotides encoding transmembrane (Domain II) and the nucleotides encoding cytoplasmic domain (Domain I) of the murine CD40 ligand gene. Examples of such preferred chimeric accessory molecules are shown in Figure 2. An exemplary

nucleotide sequence for such a gene is SEQ ID NO: 7. In other chimeric accessory molecule ligand genes of the present invention, the nucleotides encoding the extracellular domains (Domains III and IV) of the murine CD40 ligand gene may be operatively linked to nucleotides encoding the transmembrane (Domain II) and cytoplasmic domain (Domain I) of the human CD40 ligand gene. An exemplary nucleotide sequence for such a gene is SEQ ID NO: 3. In other preferred chimeric accessory molecule ligand genes of the present invention, the nucleotides encoding the extracellular domains (Domains III and IV) and transmembrane domain (Domain II) of human CD40 ligand are coupled to the nucleotides encoding cytoplasmic domain (Domain I) of murine CD40 ligand gene. An exemplary nucleotide sequence for such a gene is SEQ ID NO: 6. Other chimeric accessory molecule genes contemplated by the present invention comprise the nucleotides encoding the extracellular domains (Domains III and IV) and transmembrane domain (Domain I) of the murine CD40 ligand gene operatively linked to the nucleotides encoding cytoplasmic domain of the human CD40 ligand gene. An exemplary nucleotide sequence for such a gene is SEQ ID NO: 5. Other chimeric accessory molecule ligand genes are contemplated by the present invention in which the human CD40 ligand gene extracellular domains (Domain III and IV) is operatively linked to the murine CD40 ligand gene transmembrane domain (Domain I) which is operatively linked to the human CD40 ligand gene cytoplasmic domain (Domain I). An exemplary nucleotide sequence for such a gene is SEQ ID NO: 4.

One of ordinary skill in the art will understand that many more combinations which utilize domains or other selected segments of any of the accessory molecule ligand genes including the human CD40 ligand genes and the mouse CD40 ligand genes are possible. Such additional chimeric accessory molecule genes would

include the following genes: chimeric accessory molecule genes in which the nucleotides encoding Domain I are selected from a particular accessory molecule ligand gene and operatively linked, either directly or
5 by an additional nucleotide sequence to the nucleotides encoding Domain II from a particular accessory molecule ligand gene. These domains then would be operatively linked either directly or by an additional nucleotide sequence to the nucleotides encoding Domain III from a
10 particular accessory molecule ligand gene. This molecule would then be operatively linked either directly or by an additional nucleotide sequence to the nucleotides encoding Domain IV of a particular accessory molecule ligand gene. The chimeric accessory molecule
15 ligand gene constructed in this manner may have additional nucleotides on either end or between domains which are useful to provide different amino acids in these positions. One of ordinary skill in the art will understand that these particular combinations are merely
20 illustrations and that numerous other combinations could be contemplated in which gene segments comprising nucleotides encoding less than the entire domain of an accessory molecule are exchanged between different accessory molecules.

25 The present invention also contemplates chimeric accessory molecule ligand genes which are comprised of gene segments of mouse or human CD40 ligand in combination with gene segments derived from Fas ligand, TNF_{α} , TNF_{β} , CD70, CD30L, 4-1BBL, nerve growth factor or
30 TNF -related apoptosis inducing ligand (TRAIL). Particularly useful chimeric accessory molecule ligand genes comprise at least one gene segment which is derived from a murine CD40 ligand gene together with gene segments or a gene segments derived from a
35 different accessory molecule ligand gene.

The present invention also contemplates chimeric accessory molecule ligand genes in which the accessory

molecules produced have been modified to remove amino acids within the chimeric accessory molecule that are used by post-translational mechanisms to regulate the level of expression of the accessory molecule or
5 accessory molecule protein on a particular cell. The sites removed from the chimeric accessory molecules or chimeric molecule may include amino acids or sites which make up protease cleavage sites including
10 metallothionine proteases, serine proteases and other proteases that recognize an amino acid sequence either specifically or nonspecifically. In particular preferred embodiments, amino acids in Domain III which make up potential or actual recognition site(s) used by post-translational regulatory mechanisms have been
15 modified or removed.

The present invention also contemplates chimeric accessory molecule ligand genes in which the domains, subdomain fragments or other amino acid residues have been taken from one accessory molecule ligand gene and
20 moved into a second accessory molecule ligand gene from the same species. For example, in this particular embodiment, the human Domain I, and the human Domain II from the CD40 ligand molecule may be operatively linked to the nucleotides encoding the human Domain III from,
25 for example, the CD70 molecule which is in turn operatively linked to human Domain IV for the CD40 ligand molecule. This chimeric accessory molecule therefore contains human CD40L Domains I, II and IV and human CD70 Domain III. An exemplary nucleotide sequence
30 for such a gene is SEQ ID NO: 19. One of ordinary skill in the art will understand that a number of such combinations using domains from the same species from different accessory molecule ligand genes may create a number of chimeric accessory molecule genes which may
35 all have specific activities and properties.

The present invention contemplates chimeric accessory molecule ligand genes in which the Domain III

of a particular accessory molecule ligand gene has been replaced with a Domain III from a different accessory molecule ligand gene. In one particularly preferred embodiment, the mouse Domain III has been used to
5 replace the human Domain III in the CD40 ligand molecule. This chimeric accessory molecule therefore contains the human CD40L Domain I, the human CD40L Domain II, mouse CD40L Domain III, and human CD40L Domain IV. An exemplary nucleotide sequence for such a
10 gene is SEQ ID NO: 20.

The present invention also contemplates the use of chimeric accessory molecules that contain man-made amino acid sequences inserted into or in place of a portion of a domain or other amino acid sequence of an accessory
15 molecule gene. These man-made amino acid segments may be created by selecting any amino acid sequence that may be used to give the accessory molecule a particular function or to remove another undesired function. These man-made amino acid segments are produced by inserting
20 into the accessory molecule ligand gene or chimeric accessory molecule ligand gene the nucleotide sequences required to encode those particular man-made amino acid segments in the desired positions. Further, the chimeric accessory molecule ligand genes may contain
25 nucleotide segments which comprise sub-domain segments of other molecules or small segments in which amino acids have been changed for a desired purpose. The use of sub-domain nucleotide segments allows the introduction of short amino acid sequences derived from
30 other molecules into chimeric accessory molecules of the present invention. The incorporation of such short sub-domain segments or amino acid changes into the accessory molecule ligand allows the introduction of desired or the removal of undesired features of that molecule.

35 The identification of domain structures within accessory cell molecules is well known in the art and generally requires the identification of cysteine

residues within the accessory molecules and the subsequent mapping of disulfide bonds between various cysteine residues. The mapping of various sub-domain segments of an accessory molecule is well known in the art and involves analysis of the amino acid sequence of the accessory molecules and generally involves a comparison of the crystal structure of tissue necrosis factor with the use of predictive algorithms thereby producing a predicted structure of a chimeric accessory molecule or an accessory molecule. This predicted structure of these molecules can then be used to select various sub-domain portions of the molecule to be used to construct further chimeric accessory molecules. Examples of such mapping studies include the studies by M. Pitsch and C. V. Jongeneel, International Immunology, 5:233-238 (1993) and the analysis shown in Figure 1.

The present invention also contemplates accessory molecule ligand genes and chimeric accessory molecule ligand genes which are truncated and encode less than the full length of the amino acid sequence found in the native accessory molecule ligand. These truncations may alter the properties of the accessory molecule ligand gene but some identified activity is maintained. Such truncations may be made by removing a gene segment or gene segments from the accessory molecule gene and typically would be performed by removing nucleotides encoding domains which are not directly involved in the binding of the accessory molecule ligand with its accessory molecule. These truncated accessory molecule ligand genes or chimeric truncated accessory molecule ligand genes may contain further gene segments which encode amino acid segments or domains which replace the domains removed from that truncated accessory molecule gene. However, such replacement of the portions of the accessory molecule removed by truncation is not necessary.

The chimeric accessory molecule genes of the present invention may be constructed using standard genetic engineering methods to operatively link a particular nucleotide sequence from one accessory molecule ligand gene to a different nucleotide sequence derived from the same or different accessory molecule ligand gene. In addition, standard genetic engineering methods may be used to insert man-made nucleotide sequences or sub-domain nucleotide sequences into the chimeric accessory molecule ligand gene. One of ordinary skill in the art will understand that various methods may be utilized to produce such chimeric accessory molecule genes. For example, a gene conversion method known as "SOEN" may be used to produce a chimeric accessory molecule gene which contains nucleotide segments derived from different chimeric accessory molecules. The methods for using this gene conversion method are well known in the art and have been described for example in Horton, R. M., Mol. Biotechnol., 3:93 (1995); Ali, S. A. and A. Steinkasserer, Biotechniques, 18:746 (1995); Vilardaga, J. P., E. Di Paolo, and A. Bollen, Biotechniques, 18:604 (1995); Majumder, K., F. A. Fattah, A. Selvapandiyan, and R. K. Bhatnagar, PCR. Methods Appl., 4:212 (1995); Boles, E. and T. Miosga, Curr. Genet. 28:197 (1995); Vallejo, A. N., R. J. Pogulis, and L. R. Pease, PCR. Methods Appl., 4:S123 (1994); Henkel, T. and P. A. Baeuerle, Anal. Biochem., 214:351 (1993); Tessier, D. C. and D. Y. Thomas, Biotechniques, 15:498 (1993); Morrison, H. G. and R. C. Desrosiers, Biotechniques, 14:454 (1993); Cadwell, R. C. and G. F. Joyce, PCR. Methods Appl., 2:28 (1992); and, Stappert, J., J. Wirsching, and R. Kemler, Nucleic Acids Res., 20:624 (1992). Alternatively, one of ordinary skill in the art will understand that site-directed mutagenesis may be used to introduce changes into a particular nucleotide sequence to directly produce or indirectly be used to

produce a chimeric accessory molecule gene of the present invention. For example, the mutagen kit provided by BioRad Laboratories may be used together with the methods and protocols described within that kit to produce the desired changes in the nucleotide sequence. These methods were originally described by Kunkel, Proc. Natl. Acad. Sci. USA, 82:488-492 (1985) and Kunkel et al., Meth. Enzol. Mol., 154:367-382 (1987). By using the site directed mutagenesis protocols described herein and known within the art, a skilled investigator may induce individual nucleotide changes which result in an altered amino acid sequence or which preserve an amino acid sequence but introduce a desired restriction enzyme recognition sequence into the gene. This new restriction endonuclease recognition site may then be used to cut the gene at that particular point and use it to a gene or segment of another accessory molecule ligand gene. In addition to these methods, one of ordinary skill in the art will understand that an entire chimeric accessory molecule ligand gene may be synthesized using synthetic methods known in the art. This methodology only requires that the skilled artisan generating nucleotide sequence of a chimeric accessory molecule ligand gene and provide that sequence to a company which is capable of synthesizing such a gene.

B. Genetic Constructs

The present invention contemplates the use of accessory molecule ligand genes or chimeric accessory molecule ligand genes which are present in various types of genetic vectors. A genetic vector refers to a DNA molecule capable of autonomous replication in a cell into which another DNA segment can be inserted to cause the additional DNA segments to replicate. Vectors capable of expressing genes contained in that vector are referred to as "expression vectors." Thus, the genetic vectors and expression vectors of the present invention

are recombinant DNA molecules which comprise at least two nucleotide sequences not normally found together in nature.

The genetic vectors useful in the present invention
5 contain an accessory molecule ligand gene which encodes an accessory molecule ligand which is optionally operatively linked to a suitable transcriptional or translational regulatory nucleotide sequence, such as one derived from a mammalian, microbial, viral, or
10 insect gene. Such regulatory sequences include sequences having a regulatory role in gene expression, such as a transcriptional promoter or enhancer, an operator sequence to control transcription, a sequence encoding a ribosomal binding site within the messenger
15 RNA and appropriate sequences which control transcription, translation initiation or transcription termination.

Particularly useful regulatory sequences include the promoter regions from various mammalian, viral,
20 microbial, and insect genes. The promoter region directs an initiation of transcription of the gene and causes transcription of DNA through and including the accessory molecule ligand gene. Useful promoter regions include the promoter found in the Rous Sarcoma Virus
25 (RSV) - long terminal repeat (LTR), human cytomegalovirus (HCMV) enhancer/promoter region lac promoters, and promoters isolated from adenovirus, and any other promoter known by one of ordinary skill in the art would understand to be useful for gene expression in
30 eukaryotes, prokaryotes, viruses, or microbial cells. Other promoters that are particularly useful for expressing genes and proteins within eukaryotic cells include mammalian cell promoter sequences and enhancer sequences such as those derived from polyoma virus,
35 adenovirus, simian virus 40 (SV40), and the human cytomegalovirus. Particularly useful are the viral early and late promoters which are typically found

adjacent to the viral origin of replication in viruses such as the SV40. Examples of various promoters which have been used in expression vectors have been described by Okima and Berg (Mol. Cell. Biol. 3:280, 1983), the
5 pMLSVN SV40 described by Kossman et al., Nature 312:768 (1984). One of ordinary skill in the art will understand that the selection of a particular useful promoter depends on the exact cell lines and the other various parameters of the genetic construct to be used
10 to express the accessory molecule ligand gene or the chimeric accessory molecule ligand gene within a particular cell line. In addition, one of ordinary skill in the art will select a promoter which is known to express genes in the target cell at a sufficiently
15 high level to be useful in the present invention.

The genetic vectors and expression vectors of the present invention optionally contain various additional regulatory sequences including ribosome binding sites which allow the efficient translation of the messenger
20 RNA produced from an expression vector into proteins, the DNA sequence encoding various signals peptides which may be operatively linked to the accessory molecule ligand gene or the chimeric accessory molecule ligand gene. The signal peptide, if present, is expressed as a
25 precursor amino acid which enables improved extra-cellular secretion of translation fusion polypeptide.

The genetic constructs contemplated by the present invention therefore include various forms of accessory molecule ligand genes described above which are
30 operatively linked to either a promoter sequence or a promoter and enhancer sequence and also operatively linked to a polyadenylation sequence which directs the termination and polyadenylation of messenger RNA. It is also contemplated that the genetic constructs of the
35 present invention will contain other genetic sequences which allow for the efficient replication and expression of that construct within the desired cells. Such

sequence may include introns which are derived from native accessory molecule ligand genes or, for example, from a virus gene.

The present invention also contemplates gene therapy vectors which are able to directly infect mammalian cells so as to introduce the desired accessory molecule ligand gene or chimeric accessory molecule ligand gene into that cell. These gene therapy vectors are useful for directly infecting cells which have been isolated from an animal or patient, or can be directly introduced into an animal or patient and thereby directly infect the desired cell within that animal or patient.

Many types of gene therapy vectors which are able to successfully transfer genes and cause the expression of desired foreign DNA sequences have been developed and described in the literature. For example, the article entitled "Gene Transfer Vectors for Mammalian Cells" in Current Comm. Mol. Biol., Cold Springs Harbor Laboratory, New York (1987). Further, naked DNA can be physically introduced into eukaryotic cells including human cells by transfection using any number of techniques including calcium phosphate transfection (Berman et al., Proc. Natl. Acad. Sci. USA, 81:7176 (1984)), DEAE-Dextran Transfection, protoplast fusion (Deans et al., Proc. Natl. Acad. Sci. USA, 81:1292 (1984)), electroporation, liposome fusion, polybrene transfection and direct gene transfer by laser micropuncture of the cell membrane. In addition, one of ordinary skill in the art will understand that any technique which is able to successfully introduce the DNA into a cell in such a manner as to allow it to integrate into the genome of a cell and allow the expression of the desired gene would be useful in the present invention.

Specifically, gene therapy vectors which utilize recombinant infectious virus particles for gene delivery

have been widely described. See, for example, Brody, S. L. and R. G. Crystal, Ann. N. Y. Acad. Sci., 716:90 (1994); Srivastava, A., Blood. Cells, 20:531 (1994); Jolly, D., Cancer Gene Ther., 1:51 (1994); Russell, S. J., Eur. J. Cancer, 30A:1165 (1994); Yee, J. K., T. Friedmann, and J. C. Burns, Methods Cell Biol., 43 Pt A:99 (1994); Boris-Lawrie, K. A. and H. M. Temin, Curr. Opin. Genet. Dev., 3:102 (1993); Tolstoshev, P., Annu. Rev. Pharmacol. Toxicol., 33:573 (1993); and, Carter, B. J., Curr. Opin. Biotechnol., 3:533 (1992). The present invention contemplates the use of gene therapy vectors to carry out the desired methodology of the present invention by introducing a gene encoding an accessory molecule ligand gene or a chimeric accessory molecule ligand gene into the cell. Many viral vectors have been defined and used as gene therapy vectors and include virus vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated viruses, and retroviruses. One of ordinary skill in the art will understand that useful gene therapy vectors are vectors which are able to directly introduce into the target cells the DNA which encodes the accessory molecule ligand and allow that DNA to persist in the cell so as to express the accessory molecule ligand in the desired manner within the cell.

The gene therapy vectors of the present invention are useful for introducing accessory molecule ligand genes into a variety of mammalian cells including human cells. The particular cells infected by the gene therapy vector will depend on the various specifics of the vector and such vectors can be used to introduce the accessory molecule ligand genes of the present invention into hematopoietic or lymphoid stem cells, antigen presenting cells, embryonic stem cells, and other cells which are capable of presenting antigen within the immune system including cells which have CD40 on their surface. Further, such gene therapy vectors are able to

introduce a gene encoding an accessory molecule ligand gene into a human neoplastic cell such as a lymphoma, leukemia, AML, CLL, CML, AMML, CMML, breast cancer, lung cancer, ovarian cancer or any tumor capable of acting as antigen presenting cells or cells which can stimulate bystander antigen presenting cells. Further, the contemplated gene therapy vectors may be used to introduce the accessory molecule ligand genes of the present invention into cells which have been engineered to make those cells capable of presenting antigen to the immune system.

III. Cells Containing Genetic Constructs Encoding an Accessory Molecule Ligand or Chimeric Accessory Molecule Ligand

The present invention also contemplates various cells which contain the genetic constructs of the present invention. These cells contain the constructs which encode the accessory molecule ligand gene and thus contain the various genetic elements described in Section II.B. above. These cells may be microbial cells, eukaryotic cells, insect cells, and various mammalian cells including human cells. In preferred embodiments of the present invention, these cells include various neoplastic cells including human neoplastic cells. These neoplastic cells may be of any cell type and include cells of the immune system, and other blood cells. Particularly preferred are any neoplastic cells which may function as an antigen presenting cells within the immune system or which may stimulate bystander antigen presenting cells by expression of a transgenic accessory cell molecule of the present invention. Typically these neoplastic which are able to function to present antigen to the immune system have or have had an accessory molecule, such as the CD40 molecule, on the cell surface. Generally, these cells are naturally capable of presenting antigen to the immune system, but the present invention also

contemplates the introduction of accessory molecule ligand genes into a cell which is not naturally able to present antigen to the immune system but which has been genetically engineered to make that cell capable of presenting antigen to the immune system. Typically, these cells include various known cell types such as monocytes, macrophages, B cells, Langerhans cells, interdigitating cells, follicular dendritic cells or Kupffer cells and the like which have become neoplastic.

10 In addition, the present invention also contemplates cells from various carcinomas, breast, ovarian and lung cancers which contain the genetic constructs described herein. In other preferred embodiments, an accessory molecule ligand gene of the present invention is placed

15 into cells which may be injected into a treatment site such as a tumor bed or joint. For example, the accessory molecule ligand gene of the present invention may be inserted into a fibroblast cell and the accessory molecule ligand expressed on the surface of that cell.

20 The fibroblasts are then injected into the treatment site and cause the desired immuno effect due to the presence of the accessory molecule ligand on the surface of those cells. These cells stimulate other immune cells present in that treatment site (bystander cells).

25 This process then results in the desired effect on the immune system.

IV. Methods Utilizing Genetic Vectors and Constructs Containing an Accessory Molecule Ligand Gene

The present invention contemplates methods of altering the immunoreactivity of human cells using a method which includes introducing a gene encoding an accessory molecule ligand gene into the human cells so that the accessory molecule ligand encoded by that gene is expressed on the surface of those cells. The present

30 invention is useful for any human cells which participate in an immune reaction either as a target for

35

the immune system or as part of the immune system which responds to the foreign target. A large variety of methods are contemplated in which the final result is that the accessory molecule ligand gene is introduced
5 into the desired cells. These methods include ex vivo methods, in vivo methods and various other methods which involve injection of DNA, genetic vectors or gene therapy vectors into the animal or human, including injection directly into the tumor bed present in any
10 animal or human.

Ex vivo methods are contemplated wherein the cells into which the accessory molecule ligand gene is to be introduced are isolated from the animal or patient and then the gene is introduced into those isolated cells
15 using suitable methods. Examples of useful ex vivo methods have been described for example by Raper, S. E., M. Grossman, D. J. Rader, J. G. Thoene, B. J. Clark, D. M. Kolansky, D. W. Muller, and J. M. Wilson, Ann. Surg., 223:116 (1996); Lu, L., R. N. Shen, and H. E. Broxmeyer,
20 Crit. Rev. Oncol. Hematol., 22:61 (1996); Koc, O. N., J. A. Allay, K. Lee, B. M. Davis, J. S. Reese, and S. L. Gerson, Semin. Oncol., 23:46 (1996); Fisher, L. J. and J. Ray, Curr. Opin. Neurobiol., 4:735 (1994); and, Goldspiel, B. R., L. Green, and K. A. Calis, Clin.
25 Pharm., 12:488 (1993). D. Dilloo et al., in Blood 90:1927-1933 (1997), describe a method, using CD40L-activated cells, for treating B-acute lymphoblastic leukemia (ALL). They cocultured leukemia cells with fibroblasts infected with a retroviral vector encoding
30 CD40L, then injected the cell mix into mice. Such an approach, if taken in humans, would differ from that contemplated here in that the therapeutic cells are stimulated in vitro, by another cell line expressing the accessory molecule ligand. Schultze, J.L. et al., in
35 Blood 89: 3806-3816 (1997), describe a method for stimulating T-TILs (tumor-infiltrating T cells) cytotoxic for follicular lymphoma (FL) cells by exposing

them, in vitro, to FL B cells which were previously cultured with CD40L-expressing fibroblasts. They propose an adoptive immunotherapy in which T-TILS stimulated in this manner are transfused into patients.

- 5 This method also requires in vitro stimulation, of the cells to be transfused, with another cell line expressing an accessory molecule.

Following the introduction of the gene, including any optional steps to assure that the accessory molecule
10 ligand gene has been successfully introduced into those isolated cells, the isolated cells are introduced into the patient either at a specific site or directly into the circulation of the patient. In preferred embodiments of the present invention, cell surface
15 markers, including molecules such tumor markers or antigens identify the cells are used to specifically isolate these molecules from the patient. One of ordinary skill in the art will understand that such isolation methods are well known and include such
20 methodologies as fluorescence activated cell sorting (FACS), immunoselection involving a variety of formats including panning, columns and other similar methods.

The present invention also contemplates introducing the accessory molecule ligand gene into the desired
25 cells within the body of an animal or human patient without first removing those cells from the patient. Methods for introducing genes into specific cells in vivo, or within the patient's body are well known and include use of gene therapy vectors and direct injection
30 of various genetic constructs into the animal or patient. Examples of useful methods have been described by Danko, I. and J. A. Wolff, Vaccine, 12:1499 (1994); Raz, E., A. Watanabe, S. M. Baird, R. A. Eisenberg, T. B. Parr, M. Lotz, T. J. Kipps, and D. A. Carson, Proc.
35 Natl. Acad. Sci. U. S. A., 90:4523 (1993); Davis, H. L., R. G. Whalen, and B. A. Demeneix, Hum. Gene Ther., 4:151 (1993); Sugaya, S., K. Fujita, A. Kikuchi, H. Ueda, K.

Takakuwa, S. Kodama, and K. Tanaka, Hum. Gene Ther., 7:223 (1996); Prentice, H., R. A. Kloner, Y. Li, L. Newman, and L. Kedes, J. Mol. Cell Cardiol., 28:133 (1996); Soubrane, C., R. Mouawad, O. Rixe, V. Calvez, A. Ghoumari, O. Verola, M. Weil, and D. Khayat, Eur. J. Cancer, 32A:691 (1996); Kass-Eisler, A., K. Li, and L. A. Leinwand, Ann. N. Y. Acad. Sci., 772:232 (1995); DeMatteo, R. P., S. E. Raper, M. Ahn, K. J. Fisher, C. Burke, A. Radu, G. Widera, B. R. Claytor, C. F. Barker, and J. F. Markmann, Ann. Surg., 222:229 (1995); Addison, C. L., T. Braciak, R. Ralston, W. J. Muller, J. Gauldie, and F. L. Graham, Proc. Natl. Acad. Sci. U. S. A., 92:8522 (1995); Hengge, U. R., P. S. Walker, and J. C. Vogel, J. Clin. Invest., 97:2911 (1996); Felgner, P. L., Y. J. Tsai, L. Sukhu, C. J. Wheeler, M. Manthorpe, J. Marshall, and S. H. Cheng, Ann. N. Y. Acad. Sci., 772:126 (1995); and, Furth, P. A., A. Shamay, and L. Hennighausen, Hybridoma, 14:149 (1995). In a typical application, a gene therapy vector containing an accessory molecule ligand gene is introduced into the circulation or at a localized site of the patient to allow the gene therapy vector to specifically infect the desired cells. In other preferred embodiments the gene therapy vector is injected directly into the tumor bed present in an animal which contains at least some of the cells into which the accessory molecule ligand gene is to be introduced.

The present invention also contemplates the direct injection of DNA from a genetic construct which has a promoter and accessory molecule ligand gene followed by a polyadenylation sequence into a patient or animal. Examples of such useful methods have been described by Vile, R. G. and I. R. Hart, Ann. Oncol., 5 Suppl 4:59 (1994). The genetic construct DNA is directly injected into the muscle or other sites of the animal or patient or directly into the tumor bed of the animal or patient. Alternatively, DNA from a genetic construct containing

at least an accessory molecule ligand gene is used and directly injected into the animal.

In preferred embodiments of the present invention, the immune reaction or response of a human patient or
5 animal is altered by introducing the accessory molecule ligand gene into cells, including human cells which have an accessory molecule present on the cell surface. Such cells include human cells, human antigen presenting cells and optionally these cells may be neoplastic
10 antigen presenting cells which have the capacity to express the accessory molecule on the surface of the cell or cells which are capable of stimulating. In some embodiments, the amount of accessory molecule present on the surface of the cells into which the accessory
15 molecule ligand gene is to be introduced is very small and such small amounts of the accessory molecule may result from down-regulation of that accessory molecule on the surface of such cells. In some embodiments, the cells into which the accessory molecule ligand gene is
20 introduced have at least low levels of the CD40 molecule present on the cell surface or are derived from cells which did express the CD40 ligand molecule on the cell surface but have reduced or eliminated that expression.

The preferred methods of altering the immuno-
25 reactivity of a particular cell are applicable to mammalian cells including human cells. These human cells may include neoplastic human cells such as human lymphomas, leukemias, and other malignancies including breast, lung and ovarian cancers. In some preferred
30 embodiments the cells are normal antigen presenting cells of a human patient such as monocytes, macrophages, B cells, Langerhans cells, interdigitating cells, follicular dendritic cells, Kupffer cells, and other similar cells. In preferred embodiments, the cells are
35 lymphocytes which acquire altered immunoreactivity when the accessory molecules of the present invention are introduced into those cells. In other preferred

embodiments, the cells may be neoplastic or normal cells which are capable of stimulating bystander antigen presenting cells when the accessory molecule ligand genes of the present invention are introduced into these
5 cells. The present invention also contemplates that cells which are not naturally capable of presenting antigen to the immune system may be genetically engineered to introduce the genes encoding the molecules required for antigen presentation, including genes
10 encoding an accessory molecule, and thus allow these cells to act as artificial antigen presenting cells. The accessory molecule ligand gene may then be introduced into these artificial antigen presenting cells. Various tests are well known in the literature
15 to determine whether a particular cell is able to function as an antigen presenting cell, such as cell proliferation or the production of lymphokines and therefore this aspect of the present invention may be easily determined.

20 In addition to the above normal human cells, the present invention also contemplates introducing the accessory molecule ligand gene into various neoplastic or malignant cells which optionally are antigen presenting cells. Such human neoplastic cells which are
25 contemplated include leukemias, lymphomas, AML, AMML, or CMML, CML, CLL and any neoplastic cell which is capable of stimulating bystander antigen presenting cells when an accessory molecule ligand is introduced into that cell. Also contemplated are neoplastic cells such as a
30 breast, ovarian or lung cancer cell which is capable of or is engineered to act as an antigen presenting cell. However, the present immunomodulation also applicable to other malignancies not specifically identified and thus would include any tumor of any cell capable of
35 presenting antigen within the animal or human immune system or any cell which is capable of acting as an antigen presenting cell or capable of stimulating

bystanding antigen presenting cells after an accessory molecule ligand gene has been introduced into those cells. Generally these antigen presenting cells have accessory molecules on the surface of the cells.

5 The present methods of altering the immunoreactivity of a human or animal cell contemplate the introduction of an accessory molecule ligand gene into the cells for which altered immunoreactivity is desired. The genes useful in the present invention
10 include the wide range of accessory molecule ligand genes and chimeric accessory molecule ligand genes identified above and in preferred embodiments include at least a portion of the murine CD40 ligand gene. In particularly preferred embodiments, the accessory
15 molecule ligand gene introduced into the cells using the methods of the present invention is selected to correspond to the accessory molecule present on the surface of the cells for which altered immunoreactivity is desired. In one particular application of the
20 present invention, the immunoreactivity of a cell which expresses the CD40 molecule on the cell surface would be accomplished by introducing the gene which encodes the CD40 ligand molecule and more preferably the murine CD40 ligand molecule.

25 The present invention also contemplates altering the immunoreactivity of human or animal cells by introducing an accessory molecule ligand gene which is a chimeric accessory molecule ligand gene into the cell. The various useful chimeric accessory molecule ligand
30 genes were identified above and could include a wide variety of molecules and allow the unique properties of those chimeric accessory molecule ligand genes to be utilized to alter the immunoreactivity of the target cells. In preferred embodiments, useful chimeric
35 accessory molecule ligand genes are genes which encode at least a portion of the accessory molecule ligand which is capable of binding the accessory molecule

present on the surface of the cells for which altered immunoreactivity is desired.

The methods of the present invention for altering the immunoreactivity contemplate the use of genetic
5 vectors and genetic constructs including gene therapy vectors which encode an accessory molecule ligand and therefore contain an accessory molecule ligand gene. Typically, the genetic vectors and genetic constructs including the gene therapy vectors of the present
10 invention have a promoter which is operatively linked to the accessory molecule ligand gene followed by a polyadenylation sequence. In other embodiments, the only requirement is that the genetic vectors, genetic constructs, and gene therapy vectors of the present
15 invention contain the accessory molecule ligand gene or the chimeric accessory molecule ligand gene.

V. Methods of Treating Neoplasia

The present invention also contemplates methods of treating human neoplasia comprising inserting into a
20 human neoplastic cell a gene which encodes an accessory molecule ligand so that the accessory molecule ligand is expressed on the surface of the neoplastic cells. The present invention contemplates treating human neoplasia both in vivo, ex vivo and by directly injecting various
25 DNA molecules containing a gene which encodes an accessory molecule ligand into the patient. However, at a minimum, the present methods for treating human neoplasia involve inserting the gene encoding the accessory molecule ligand into the neoplastic cells in
30 such a way as to allow those neoplastic cells to express the accessory molecule ligand on the cell surface. The expression of the accessory molecule ligand gene in these neoplastic cells modulates the immune system to cause the neoplasia to be reduced or eliminated.

35 In a preferred method of treating human neoplasia, the method further comprises the steps of first

obtaining the human neoplastic cells from a human patient and then inserting into the isolated human neoplastic cells a gene which encodes an accessory molecule ligand so that the accessory molecule ligand is
5 expressed on the surface of the neoplastic cells. The human neoplastic cells having the accessory molecule ligand on the surface of that cell are then infused back into the human patient. One of ordinary skill in the art will understand that numerous methods are applicable
10 for infusing the altered human neoplastic cells containing the gene encoding the accessory molecule ligand back into the patient and that these methods are well known in the art.

The contemplated methods of treating human
15 neoplasia are applicable to a wide variety of human neoplasias including lymphomas, leukemias, and other malignancies. In preferred embodiments the human neoplasia is a neoplasia which involves the antigen presenting cells of the human immune system and includes
20 monocytes, macrophages, B cells, Langerhans cells, interdigitating cells, follicular dendritic cells, Kupffer cells, and the like. In other preferred embodiments, the human neoplasia is a leukemia, a lymphoma, AML, AMML, CMML, CML or CLL, lung cancer,
25 breast cancer, ovarian cancer and other similar neoplasias.

The genetic vectors, genetic constructs and gene therapy vectors useful in the methods of treating human neoplasia of the present invention have been disclosed
30 above and include constructs in which a promoter is operatively linked to the accessory molecule ligand gene or the chimeric accessory molecule ligand gene which is in turn operatively linked to a polyadenylation sequence. The methods of treating human neoplasia
35 contemplate the use of genetic constructs, genetic vectors and gene therapy vectors as described in this specification. In addition, the present invention

contemplates the use of DNA which contains at least a gene encoding an accessory molecule ligand gene. This gene may or may not contain a promoter and other regulatory sequences.

5 In preferred embodiments of the present invention, the cells comprising the human neoplasia are located in at least one defined site termed a tumor bed within the human patient. This tumor bed typically contains the tumor or neoplastic cell together with a number of other
10 cells which are associated with the tumor or neoplastic cells. The present invention contemplates methods of treating such human neoplasia present in a tumor bed by injecting into the tumor bed of the patient, a gene which encodes an accessory molecule ligand so that the
15 accessory molecule ligand is expressed on the surface of the tumor cells thereby causing the cells to participate in an immune reaction. The gene which encodes the accessory molecule ligand may be present as part of a gene therapy vector, genetic construct or genetic
20 vector.

In preferred embodiments, the accessory molecule ligand gene is a chimeric accessory molecule ligand gene which has at least a portion of the murine CD40 ligand gene is used. In other preferred embodiments, the
25 accessory molecule ligand encoded is capable of binding an accessory molecule present on the human neoplasia to be treated.

The various gene therapy vectors used in the treatment methods of the present invention include
30 vectors which are capable of directly infecting human cells. Such vectors have been described in the literature and are readily adaptable to the methods described in the present invention.

The present invention contemplates the use of any
35 type of gene therapy including the methods of Raper, S.E. et al., Ann. Surg., 223:116 (1996); Lu, L. et al., Crit. Rev. Oncol. Hematol., 22:61 (1996); Koc, O. N. et

- al., Semin. Oncol., 23:46 (1996); Fisher, L. J. et al., Curr. Opin. Neurobiol., 4:735 (1994); Goldspiel, B. R. et al., Clin. Pharm., 12:488 (1993); Danko, I. et al., Vaccine, 12:1499 (1994); Raz, E. et al., Proc. Natl. Acad. Sci. U.S.A., 90:4523 (1993); Davis, H. L. et al., Hum. Gene Ther., 4:151 (1993); Sugaya, S. et al., Hum. Gene Ther., 7:223 (1996); Prentice, H. et al., J. Mol. Cell Cardiol., 28:133 (1996); Soubrane, C. et al., Eur. J. Cancer, 32A:691 (1996); Kass-Eisler, A. et al., ann. N. Y. Acad. Sci., 772:232 (1995); DeMatteo, R. P. et al., Ann. Surg., 222:229 (1995); Addison, C. L. et al., Proc. Natl. Acad. Sci. U.S.A., 92:8522 (1995); Hengge, U. R. et al., J. Clin. Invest., 97:2911 (1996); Felgner, P. L. et al., Ann. N. Y. Acad. Sci., 772:126 (1995); Furth, P.A., Hybridoma, 14:149 (1995); Yovandich, J. et al., Hum. Gene Ther., 6:603 (1995); Evans, C.H. et al., Hum. Gene Ther., 7:1261.

VI. Methods of Vaccination

- The present invention contemplates methods of
20 vaccinating an animal against a predetermined organism comprising administering to that animal a vaccine containing immunogenic animal antigens capable of causing an immune response in that animal against the desired organism together with a vector containing a
25 gene encoding an accessory molecule ligand. The present invention also contemplates methods of vaccinating an animal which include administering the genes which encode the immunogenic antigen capable of causing a desired immune response or altering the immune response
30 to a particular antigen together with a vector containing a gene including the accessory molecule ligand gene. In this particular embodiment, the vector or vectors introduced encode the immunogenic antigens desired and the desired accessory molecule ligand. The
35 present invention also contemplates that the gene or genes encoding the immunogenic peptide or peptides may

be present on the same vector as is the gene or genes encoding the accessory molecule ligand.

The vaccination methods of the present invention are general in that they may be used to produce a
5 vaccination against any predetermined organism, such as a virus, a bacteria, a fungus or other organism. In addition, the present vaccination methods may be used to produce an immune response against a neoplastic cell.

In other preferred embodiments, the vaccination
10 methods of the present invention utilize a genetic vector, a genetic construct or a gene therapy vector which contains an accessory molecule ligand gene which is a chimeric accessory molecule ligand gene. That chimeric accessory molecule ligand gene preferably
15 contains at least a portion of the murine CD40 ligand gene. In other preferred embodiments, the vaccination method utilizes a DNA molecule which encodes at the minimum the accessory molecule ligand gene or a chimeric accessory molecule ligand gene. This particular DNA may
20 or may not include a promoter sequence which directs the expression of the accessory molecule ligand gene.

The present invention also contemplates that the vaccination method may utilize a genetic vector which is capable of expressing an accessory molecule ligand
25 within a particular cell or organism together with a vector which is capable of expressing at least a single polypeptide from an andovirus. This andovirus polypeptide may be expressed from the same or different vector which expresses the accessory molecule ligand in
30 that cell. In this particular embodiment, the andovirus polypeptide is also expressed in at least one cell type within the organism and serves to modulate the immune response found in response to this vaccination protocol.

The present invention also contemplates the
35 introduction of an accessory molecule ligand gene into cells which are present in the joints of patients with rheumatoid arthritis. In preferred embodiments, the

accessory molecule ligand gene introduced comprises at least a portion of the Fas ligand gene and upon expression the accessory ligand induces the cell death of cells expressing Fas on the cell surface. This process leads to the reduction of the destructive inflammatory process.

The following examples are provided to illustrate various aspects of the present invention and do not limit the scope of that invention.

10 VII. Methods of Treating Arthritis

The present invention also contemplates methods of treating arthritis comprising inserting into a joint, cells which have been transformed with an accessory molecule, such as the Fas ligand. In preferred embodiments, the expression of that accessory molecule ligand or the stability of that molecule on the surface of the cells has been altered. In these preferred embodiments, the accessory molecule ligand functions in an enhanced manner to aid in the treatment of arthritis within the joint. The present invention contemplates treating human arthritis both in vivo, ex vivo, and by directly injecting various DNA molecules containing genes which encode the useful accessory molecule ligand into the patients. Various useful protocols may be designed to rheumatoid arthritis including those described in the example section below.

The present invention contemplates the treatment of arthritis utilizing accessory molecule ligand genes which may be chimeric accessory molecule ligand genes comprised of portions of that gene being derived from two different accessory molecule ligand genes. In other embodiments, the chimeric accessory molecule ligands may be produced by utilizing domains from the same accessory molecule ligand gene. The resulting chimeric accessory molecule ligands have an altered stability on the surface of cells upon which they are expressed. This

altered stability modulates the function of the immune system in the local environment around the cells in which these chimeric accessory molecule ligands are expressed. For example, in certain preferred
5 embodiments, Fas ligand stability is altered on the surface of cells within a joint of a patient suffering from arthritis. This altered stability modulates the immune system and causes the cells to be targeted for apoptosis and thus reducing the immune response within
10 the inflamed joint. In other embodiments, the accessory molecule ligand genes described within are altered such that the resulting accessory molecule ligand has an altered stability and causes an immunomodulatory effect which can be useful in the treatment of arthritis.

15 The present invention contemplates in preferred embodiments that chimeric accessory molecule ligands genes be utilized in the treatment of arthritis. These chimeric accessory molecule ligand genes preferably contain at least a portion of the Fas ligand gene Domain
20 IV, which carries the effect or function for Fas ligand. In preferred embodiments, at least in the portion of that domain, is present which allows Fas ligand to have its biologic effects. In other preferred chimeric accessory molecule ligands, those ligands contain
25 domains from other accessory molecule ligand genes of the present invention or from a different domain of the same accessory molecule ligand. Particularly preferred are Fas chimeric accessory molecule ligand genes made up on Domain IV of the human Fas ligand operatively linked
30 with Domain III of the mouse Fas ligand. This particular combination results in more stable Fas ligand and thus, by replacing Domain III of human Fas ligand with Domain III of the mouse ligand, the activity of the human Fas ligand gene is altered.

35 Alternatively, in other preferred embodiments, the murine Fas ligand gene is used to encode the murine Fas ligand on the surface of cells in place of the human Fas

ligand. The murine Fas ligand is more stable than the human Fas ligand and thus, alters the Fas ligand activity in the joint. The resulting alter Fas ligand activity is useful in the treatment of rheumatoid arthritis.

Further preferred embodiments include embodiments in which the effect or function present on Domain IV of the human Fas ligand is combined with other domains from other accessory molecule ligands. For example, CD70 Domain III is more stable than Domain III of the human Fas ligand and thus the chimeric accessory molecule ligand made up of Domain III from the human CD70 and Domain IV of the Fas ligand together with other supporting domains would be more stable. The increased stability leads to increase Fas ligand activity. In other preferred embodiments, Domain III of the Fas ligand is replaced with multiple copies of a domain or domains. Such multiple copies of domains include domains made up of two or more copies of other domains such as Domains III or I of the CD70 molecule.

In other preferred embodiments, the present invention contemplates accessory molecule ligand genes, such as Fas ligand genes, in which a cleavage site for matrix-metalloproteinase (MMP), have been removed from the accessory molecule ligand. MMP cleavage and recognition sites, charted in Figure 28, are discussed in Smith, M.M. et al., Journal of Biol. Chem. 270:6440-6449 (95) and Nagase, H., and G.B. Fields, Biopolymers (Peptide Science) 40:399-416 (96). In preferred embodiments, at least one MMP site has been removed from at least Domain III of the Fas ligand gene. The removal of the MMP site from the Fas ligand gene makes the Fas ligand more stable and thus, more effective in the treatment of arthritis.

In other preferred embodiments, chimeric accessory molecule ligand genes are comprised of portions of the human Fas ligand gene with other domains from other

human accessory molecule ligands or domains from accessory molecules derived from other species. For example, the present invention contemplates the use of domains from CD40 ligand, CD70 ligand, CD30 ligand, TNF-related apoptosis inducing ligand (TRAIL), TNF- α as well as mutants of human Fas ligand and murine Fas ligand. Production of such chimeric accessory molecule ligands is easily accomplished by manipulating and producing accessory molecule ligand genes which are chimeric and thus has portions derived from at least two different accessory molecule ligand genes.

EXAMPLES1. Expression of Human and Mouse Accessory Molecule
Ligand in Human CLL Cells5 a. Construction of a Genetic Construct and Gene
Therapy Vector Containing a Human and Mouse
Accessory Molecule Ligand Gene

Either the human accessory molecule ligand gene
(human CD40 ligand) or the murine accessory molecule
ligand gene (murine CD40 ligand) was constructed
10 utilizing the respective human and murine genes. Each
of these genes was cloned in the following manner.

i. Murine CD40-L cloning

Total RNA was isolated using the RNA STAT-60 kit
(Tel-Test "B" Inc., Friendswood, TX) from 1 x 10⁷ B6
15 mouse splenocytes that were previously activated for 8
hours with immobilized CD3-specific mAb. cDNA was then
synthesized with the Superscript cDNA synthesis kit
(Gibco BRL, Grand Island, NY) using oligo-dT primers.
The murine CD40 ligand (mCD40-L) gene was then amplified
20 from the cDNA by PCR using the following mCD40-L
specific primers. 5'-GTTAAGCTTTTCAGTCAGCATGATAGAA (SEQ
ID NO: 26), 5'-GTTTCTAGATCAGAGTTTGAGTAAGCC (SEQ ID NO:
27). The amplified mCD40-L PCR product was subcloned
into the HindIII and XbaI sites of the eukaryotic
25 expression vector pcDNA3 (Invitrogen, San Diego, CA). A
DNA fragment encompassing the CMV promoter, mCD40-L
gene, and polyadenylation signal was released from this
plasmid construct after restriction digestion with BglII
and XhoI enzymes. This DNA fragment was then subcloned
30 into the shuttle plasmid MCS(SK)pXCX2 (Spessot R, 1989,
Virology 168:378) that was designated mCD40-L pXCX2.
This plasmid was used for adenovirus production as
described below.

ii. Human CD40-L Cloning

35 A plasmid containing the gene for human CD40-L was
used to produce the human CD40-L gene used herein. The

sequence of this gene is available and thus this source of the gene was used merely for convenience. See GenBank accession no. X67878. This plasmid was used for PCR amplification of the human CD40-L gene using the
5 specific primers, sense primer 5' CCAAGACTAGTTAACACAGCATGATCGAAA 3' (SEQ ID NO: 28) and antisense primer 5' CCAATGCGGCCGCACTCAGAATTCAACCTG 3' (SEQ ID NO: 29).

These primers contain flanking restriction enzyme
10 sites for subcloning into the eukaryotic expression plasmid pRc/CMV (Invitrogen). The PCR amplified CD40-L fragment was subcloned into the SpeI and NotI sites of pRc/CMV and designated hCD40-L pRc/CMV. A BglII and XhoI fragment encompassing the CMV promoter, hCD40-L
15 gene, and polyadenylation signal was then released from this plasmid and subcloned into the shuttle plasmid MCS(SK)pXCX2 as described above. This plasmid was designated hCD40-L pXCX2. This plasmid was used for adenovirus production as described below.

20 iii. Adenovirus Synthesis

Either mCD40-L pXCX2 or hCD40-L pXCX2 plasmids were co-transfected with pJM17 (Graham and Prevec, 1991, Methods in Molecular Biology, Vol 7) into 293 cells (American Type Culture Collection, Rockville, MD) using
25 the calcium phosphate method (Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning, A Laboratory Manual, 2nd edition, chapter 16:33-34). Isolated adenovirus plaques were picked and expanded by again infecting 293 cells. High titer adenovirus preparations were obtained
30 as described (Graham and Prevec, 1991, Methods in Molecular Biology, Vol 7), except for the following modifications. The cesium chloride gradient used for concentrating viral particles was a step gradient, with densities of 1.45 g/cm³ and 1.2 g/cm³. The samples were
35 spun in a SW41 rotor (Beckman, Brea, CA) at 25,000 rpm at 4°C. The viral band was desalted using a Sephadex

G25 DNA grade column (Pharmacia, Piscataway, NJ). The isolated virus was stored at 70°C in phosphate buffered saline with 10% glycerol. The virus titer was determined by infecting 293 cells with serial dilutions of the purified adenovirus and counting the number of plaques formed. Viral titers typically ranged from 10^{10} to 10^{12} plaque forming units/ml (PFU/ml).

10 b. Introduction of a Murine and Human Accessory Molecule Ligand Gene into CLL Cells and HeLa Cells

For adenovirus infection, 10^6 freshly thawed and washed CLL cells or HeLa cells were suspended in 0.5 to 1 mL of culture medium for culture at 37°C in a 5% CO₂-in-air incubator. Adenovirus was added to the cells at varying multiplicity of infection (MOI), and the infected cells were cultured for 48 hours, unless otherwise stated, before being analyzed for transgene expression.

20 c. Expression of an Accessory Molecule Ligand Gene in CLL Cells and HeLa Cells

The CLL and HeLa cells which were infected with the adenovirus vector containing either mouse or human CD40 ligand genes prepared in Example 1b. were then stained with commercially available monoclonal antibodies immunospecific for either human or mouse CD40 ligand (Pharmingen, San Diego, CA) using the manufacturer's directions. The CLL and HeLa cells were washed in staining media (SM) consisting of RPMI-1640, 3% fetal calf serum and 0.05% sodium azide and containing propidium iodide and then analyzed on a FACScan (Becton Dickinson, San Jose, CA). Dead cells and debris were excluded from analysis by characteristic forward and side light scatter profiles and propidium iodide staining. Surface antigen expression was measured as the mean fluorescence intensity ratio (MFIR). MFIR

equals the mean fluorescence intensity (MFI) of cells stained with a specific FITC-conjugated MoAb, divided by the MFI of cells stained with a control IgG-FITC. This method controls for the nonspecific increases in auto-
5 fluorescence seen in larger, more activated cells.

The histograms, generated for the CLL cells and HeLa cells containing either a genetic vector containing the human CD40 ligand gene or the murine CD40 ligand gene and the appropriate controls, are shown in Figure
10 3A-3D. The expression of both the murine and human accessory molecule ligand gene (CD40 ligand) in HeLa cells is shown in Figures 3A and 3B, respectively. The expression of the murine and human accessory molecule ligand in CLL cells is shown in Figures 3C and 3D. The
15 expression of an accessory molecule ligand gene in CLL cells and the expression of murine CD40 ligand on the surface of the CLL cells is shown in Figure 3C. The failure of the human accessory molecule ligand to be expressed on the surface of the CLL cells is shown in
20 Figure 3D.

Figure 8 shows data from an experiment done to examine whether the CD4⁺ T cells of CLL patients could be induced to express the accessory molecule ligand mRNA after CD3 ligation. An ELISA-based quantitative
25 competitive RT-PCR was used to measure CD40 ligand transcript levels. In this experiment, CD40 ligand and RNA transcribed from the CD40 ligand gene in CLL cells are compared with levels of CD40 ligand and RNA made in normal donor cells, after induction by CD3 ligation.
30 For CD3 activation, plate coats of CD3 mAb were made and incubated with plated CLL or normal donor mononuclear cells for the indicated amount of time, after which cells were analyzed for expression of surface antigens or CD154 RNA message levels. CLL or normal donor serum
35 was added to the cells at the beginning of the activation assay for examination of modulation of CD40 ligand surface expression.

For quantitative CD154 RT-PCR ELISA, total RNA was extracted and competitor RNA was generated from the insert containing CD40 ligand (CD154) cDNA. Varying amounts of competitor RNA were added to separate wells of isolated total RNA that subsequently were converted into cDNA. CD3 activation, ELISAs and PCR reactions were performed as described in Cantwell, M. et al., Nature Medicine 3:984-989 (1997). Biotinylated PCR products were captured onto microtiter plates (Becton Dickinson, Oxnard, CA) coated with streptavidin (Sigma), and incubated. The plate was treated with NaOH to remove the sense strands and subsequently washed. The DNA was then hybridized with either wild-type gene-specific or competitor-specific oligonucleotides. Using terminal transferase, each probe was labeled with a molecule of digoxigenin-11-dideoxyUTP (Boehringer Mannheim). The plate was incubated and washed with HYBE buffer and blocking buffer, then peroxidase-conjugated anti-digoxigenin antibody (150 U/ml; Boehringer Mannheim) in blocking buffer was added. TMB (tetramethylbenzidine) and peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD) were added for color development, and optical densities were measured at 450 nm and Deltasoft II (Biometallics, Princeton, NJ) was used for data analysis.

Standard curves plotting the moles of RNA product versus the optical density were made for the standard cDNA reactions. The equations describing these standard curves were then used to calculate the moles of wild-type or competitor DNA present in the unknown PCR reactions based on the optical densities obtained in the ELISA readings. The ratio of the quantity of wild-type DNA to the amount of competitor DNA was then plotted against the known quantity of competitor RNA added in the initial samples. The ratio of 1 was taken for the extrapolation of the amount of unknown moles of target RNA in the sample (a ratio of 1 means the amount of

target RNA versus competitor RNA are equal). The molecules of target RNA per CD4 cell was then calculated based on the following formula: [(moles target CD154 RNA) x (6 X 10²³ molecules/mole) x (dilution factor of test RNA)]/(% of CD4 T cells in total cell population).

The upper graph in Figure 8 shows that T cells of patients with CLL do not express detectable CD40 ligand after CD3 ligation. CD40 ligand RNA is produced, but it is not stable. Although both CD40 ligand and CD40 ligand RNA are expressed in normal donor T cells (lower graph), the levels of neither the protein or RNA are stably maintained.

Figure 9 shows a time course for surface expression of CD40 ligand. Expression reached a peak level at 48 hours after infection and persisted at high levels for at least 6 days thereafter. In this experiment, CLL B cells were infected with a gene therapy vector containing an accessory molecule ligand, at a MOI of 1000 at time zero, and then assessed by flow cytometry at various times thereafter. At each time point listed on the abscissa, the proportions of viable CLL B cells that expressed detectable CD154 are indicated by the vertical bars corresponding to the percentage scale depicted on the right-hand ordinate.

d. Function of the Human and Murine Accessory Molecule Ligands
i. Induction of CD80 and CD54 on Cells Containing a Gene Therapy Vector Encoding an Accessory Molecule

The CLL cells infected with the murine accessory molecule ligand gene prepared in Example 1b. were then cultured in tissue culture plates. The CLL cells were then analyzed using multiparameter FACS analysis to detect induction of CD80 and CD54 expression using fluorescein isothiocyanate-conjugated monoclonal antibodies immunospecific for each of these respective

surface antigens. Non-infected CLL cells were used as a control. The cells were subjected to the appropriate FACS analysis and histograms were generated. CD80 mAb was obtained from Dr. Edward Clark and CD54 mAb was purchased from CALTAG Inc. The CD80 was conjugated using standard methods which have been described in Kipps et al., Laboratory Immunology II, 12:237-275 (1992).

The results of this analysis are shown in Figure 4A-4D. Figures 4A-4B compare the amount of CD54 expression in CLL cells which have not been transfected (Figure 4A) or CLL cells into which a gene therapy vector containing the murine CD40 ligand gene was introduced (Figure 4B). The shaded graph indicates the isotype control for FACS staining and the open graph indicates the cells stained with the anti-CD54 antibody. These results show that the level of expression of CD54 is increased in CLL cells into which the gene therapy vector containing the murine CD40 ligand was introduced.

Figures 4C and 4D compare the amount of CD80 expression in CLL cells which have not been transfected (Figure 4C) or CLL cells into which a gene therapy vector containing the murine CD40 ligand gene was introduced (Figure 4D). The shaded graph indicates the isotype control for FACS staining and the open graph indicates the cells stained with the anti-CD80 antibody. These results show that the level of expression of CD80 is increased in the CLL cells into which the gene therapy vector containing the murine CD40 ligand was introduced.

In an additional experiment, CLL cells infected with a gene therapy vector containing the murine accessory molecule ligand gene were evaluated by flow cytometry for induced expression of not only CD54 and CD80, but also CD86, CD58, CD70 and CD95. Fluorescein-conjugated mAb specific for human CD54 and CD70 were purchased from CALTAG. Fluorescein-conjugated mAb

specific for human CD27, CD58, CD80, CD86, or CD95, and phycoerythrin-conjugated mAb specific for human or mouse CD40 ligand, were obtained from PharMingen. Shaded histograms represent staining of CLL B cells with FITC-conjugated isotype nonspecific mAb. In contrast to uninfected CLL cells (Figure 10, thin-lined histograms), or Ad-lacZ-infected CLL cells (data similar to that obtained with uninfected cells, but not shown), CLL cells infected with the adenovirus vector encoding the CD40 ligand (CD154) expressed high levels of CD54 (Figure 10, top left), CD80 (Figure 10, top middle), CD86 (Figure 10, top right), CD58 (Figure 10, bottom left), CD70 (Figure 10, bottom middle), and CD95 (Figure 10, bottom right). On the other hand, CD40 ligand-CLL (CD154 CLL) expressed significantly lower levels of both surface membrane CD27 (Figure 11A, thick-lined histogram) and soluble CD27 (Figure 11B) than uninfected (Figure 11A, thin-lined histogram) ($P < 0.01$, Bonferroni t-test) or Ad-lacZ-infected CLL cells (data similar to that obtained with uninfected cells, but not shown). In the experiment shown in Figure 11A, the CLL B cells were examined for expression of CD27 via flow cytometry, three days after infection. Shaded histograms represent staining of CLL B cells with FITC-conjugated isotype control mAb. In Figure 11B, cell-free supernatants were collected, after the infection or stimulation of CLL B cells, for 72 hours and tested for the concentration of human CD27 by ELISA. The reduced expression of CD27 (Figure 11B) is similar to that noted for leukemia B cells stimulated via CD40 cross-linking with mAb G28-5 presented by CD32-expressing L cells, as described in Rassenti, L.Z. and T.J. Kipps, J. Exp. Med. 185:1435-1445.

ii. Allogeneic T Cell Responses to CLL Cells
Into Which a Genetic Therapy Vector
Containing a Murine CD40 Ligand Gene Has
Been Introduced

5 The ability of CLL cells which have been infected
with a gene therapy vector containing the murine CD40
ligand gene to stimulate allogeneic T cells (i.e., from
another individual) was analyzed using cell prolifera-
tion assays: Briefly, the test cells were co-cultured
10 with the genetic therapy vector containing the lac-Z
gene or the murine CD40 ligand gene at a multiplicity of
infection of 1,000 in the presence of IL-4 at a con-
centration of 10 ng/ml. In other samples, the CLL cells
were stimulated with MOPC21 (a control IgG) or G28-5 (an
15 anti-CD40 monoclonal antibody) or were preincubated on
CD32-L cells and at the same time treated with IL-4.
The preincubation with the CD32-L cells together with
IL-4 treatment have been shown to be an efficient form
of cross-linking the CD40 molecule other than direct
20 gene transfection.

After three days of culture at 37°C, these cells
were treated with mitomycin C to prevent their
proliferation and then used to stimulate allogeneic T
cells. Prior to this co-culture, the different aliquots
25 of CLL cells had either been treated with the anti-CD40
monoclonal antibody or had been infected with the gene
therapy vector containing either the lac-Z or murine
CD40 ligand gene at a stimulator ratio of 1:10. After
two days of culture at 37°C, interferon gamma (IFN γ)
30 production was measured by ELISA assay. After five days
of co-culture at 37°C, the incorporation of ³H-thymidine
into replicating cells was measured after an eight hour
pulse label. The results of this assay are shown in
Table II below and in Figure 5.

35 In another experiment, CLL B cells infected with
the gene therapy vector containing the CD40 ligand gene
were evaluated for their ability to act as stimulator

cells in an allogeneic mixed lymphocyte T cell reaction (MLTR). In parallel, the stimulatory capacity of control lac-Z-vector-infected CLL cells and CLL B cells that had been cultured with CD32-L cells and an anti-
5 CD40 mAb (G28-5) or an isotype control Ig, was also examined as described in Ranheim, E.A. and T.J. Kipps, J. Exp. Med., 177:925-935 (1993), Clark, E.A. and J.A. Ledbetter, Proc. Natl. Acad. Sci. USA, 83:4494-4498 (1986), and Banchereau, J. et al., Science 251:70-72
10 (1991). Effector T cells from a non-related donor were co-cultured with the CLL stimulator cells at an effector to target ratio of 4:1. After 18 h culture at 37°C, over 30% of the allogeneic CD3⁺ cells were found to express the activation-associated antigen CD69 when
15 cultured with CD154-CLL cells (data not shown). In contrast, less than 4% of the T cells expressed CD69 when co-cultured with uninfected or Ad-lacZ-infected CLL cells (data not shown).

Two days after the initiation of the MLTR, the
20 concentrations of IFN γ in the culture supernatants were assayed by ELISA. The supernatants of the MLTR stimulated with CLL cells infected with the accessory molecule ligand CD40L (Figure 12A, CD154-CLL) contained significantly higher levels of IFN γ (306 ± 5 ng/ml, $m \pm$
25 SE, $n = 3$) than that of MLTR cultures stimulated with the anti-CD40 mAb (Figure 12A, aCD40-CLL) (23 ± 3 ng/ml) ($P < 0.05$, Bonferroni t-test). The latter was not significantly different from that of MLTR cultures stimulated with control Ad-lacZ-infected CLL cells
30 (Figure 12A, lacZ-CLL) (43 ± 10 ng/ml) ($P > 0.1$, Bonferroni t-test). The supernatants of effector cells alone, or of MLTR cultures stimulated with uninfected CLL cells (Figure 12A, CLL) or control Ig treated CLL cells (Figure 12A, MOPC-CLL), did not contain detectable
35 amounts of IFN γ (< 2 ng/ml). Similarly, none of the leukemia B cell populations produced detectable amounts

of IFN γ when cultured alone, without added effector T cells (data not shown).

After 5 days, cell proliferation was assessed by incorporation of ^3H -thymidine. Cultures with isotype control IgG-treated (Figure 12B, MOPC-CLL) or uninfected (Figure 12B, CLL) stimulator cells did not incorporate more ^3H -thymidine than cultures without added leukemia-stimulator cells (Figure 12B, None). Ad-lacZ-infected CLL B cells (Figure 12B, lacZ-CLL) also were unable to stimulate allogeneic T cells to incorporate amounts of ^3H -thymidine that were much greater than that of control cultures. In contrast, anti-CD40-stimulated leukemia cells or CD154-CLL cells each induced significant effector cell proliferation (Figure 12B, aCD40-CLL or CD154-CLL) ($P < 0.05$, Bonferroni t-test). Moreover, the amount of ^3H -thymidine incorporated by cultures stimulated with CD154-CLL cells ($41,004 \pm 761$ cpm ($m \pm \text{SE}$), $n = 3$) was significantly greater than that of cultures stimulated with equal numbers of aCD40-CLL cells ($22,935 \pm 1,892$ cpm, $n = 3$) ($P < 0.05$, Bonferroni t test). However, neither of these mitomycin-C-treated leukemia cell populations incorporated ^3H -thymidine when cultured without effector T cells (data not shown). Also, as described for the MLTR between allogeneic T cells and CD40-stimulated CLL cells {6549, 7167, 7168}, allogeneic T cell proliferation in response to CD154-CLL could be inhibited by CTLA-4-Ig or CD11a mAb when added at the initiation of the MLTR, indicating that respective interactions between CD80/CD86 and CD28, or CD54 and CD11a/CD18, contribute to the noted allogeneic T cell reaction (data not shown).

Table II

Allogeneic T cell responses to CLL cells
infected with mCD40-L adenovirus

		<u>% positive cells</u>	<u>Allogeneic response</u>		
				<u>(mean\pmSEM)</u>	
	Stimulators	mCD40-L	Human CD80	3H-TdR uptake (cpm)	IFN γ production (ng/ml)
5	None (t cells only)	-	-	3577 \pm 821	n.d.*
	CLL with:				
	No activation	0	1.4	4577 \pm 1097	n.d.
	MOPC21	0	1.0	5259 \pm 1788	n.d.
	G28-5	0	26.7	22935 \pm 1892	22.3 \pm 1.6
10	lac-Z adeno	0	4.8	9037 \pm 1781	43.2 \pm 10.5
	mCD40-L adeno	17.5	19.7	41004 \pm 761	305.7 \pm 4.5

* n.d. - not detectable

iii. Stimulation of Gamma Interferon by CLL
Cells Containing an Accessory Molecule
Ligand Gene

15 The function of CLL cells containing an accessory
molecule ligand gene (mouse CD40 ligand) was analyzed by
determining the ability of those cells to activate T
lymphocytes. The procedure was performed as follows:
20 allogeneic T lymphocytes from a healthy donor (greater
than 90% CD3⁺) were purified using magnetic beads and
monoclonal antibodies specific for the CD14 and CD19
antigen. These allogeneic T lymphocytes then were
cultured together with MMC-treated CLL cells which were
25 infected with the accessory molecule ligand gene (murine
CD40 ligand) or the lac-Z gene. This co-culture was
performed in RPMI-1640 medium containing 10% fetal calf

serum. After culture for 24 hours, the cells were collected and analyzed to determine the expression of the antigen CD69 on the T lymphocytes using a standard FACS sorting protocol. The cell culture supernatants were collected after two days in culture and tested to determine the concentration of human interferon gamma using an ELISA assay. A portion of the CLL cells containing an accessory molecule ligand gene (murine CD40 ligand) and a portion of the cells containing the adenovirus expressing the lac-Z were cultured in the presence of human interleukin 4 IL-4 (5 ng/mL). The production of interferon gamma by allogeneic T lymphocytes in the presence of this amount of human interleukin 4 was also analyzed. The results from these analyses are shown in Figure 6.

As can be seen, the human CLL cells containing the accessory molecule ligand gene (murine CD40) produced substantially higher concentrations of interferon gamma in the cell culture supernatant when compared to CLL cells which contained the lac-Z gene. The increased production of interferon gamma (IFNg) by T lymphocytes exposed to CLL cells containing the accessory molecule ligand gene indicates that these CLL cells containing the accessory molecule ligand genes were effective in producing an enhanced immune response.

iv. Stimulation of Allogeneic T Cells Pre-Exposed to Non-Modified CLL B Cells Containing an Accessory Molecule Ligand Gene

Prior studies indicated that antigen presentation to T cells, in the absence of the signals derived from costimulatory molecules such as CD28, can lead to specific T cell clonal anergy. For this reason, allogeneic T cells that had previously been cultured, with non-modified CLL B cells lacking expression of CD80 and other immune accessory molecules, were tested for

their ability to respond to CLL cells containing the CD40 ligand gene. Allogeneic effector cells did not incorporate more ^3H -thymidine in response to non-modified CLL cells (Figure 12C, CLL), or control CLL cells infected with Ad-lacZ (Figure 12C, lacZ-CLL), than when they were cultured alone (Figure 12C, None). In contrast, even after prior co-culture with non-modified CLL B cells, allogeneic effector cells could still be induced to proliferate (Figure 12C, CD154-CLL) or to produce IFN γ (Figure 12D, CD154 CLL) in response to cells expressing an accessory molecule ligand. Although modest amounts of IFN γ were detected in the supernatants of such secondary cultures when Ad-lacZ-infected leukemia cells were used as stimulator cells (Figure 12D, lacZ-CLL), this level was significantly lower than that noted for secondary cultures with Ad-CD40-ligand-infected CLL cells (Figure 12D, CD154-CLL) ($P < 0.05$, Bonferroni t-test). Similarly, the supernatants of the leukemia cells alone (data not shown), and the effector cells alone (Figure 12D, None), of the MLTR cultures stimulated with uninfected CLL cells (Figure 12D, CLL), contained negligible amounts of IFN γ (< 2 ng/ml). These results indicate that allogeneic effector cells cultured with nonmodified CLL B cells are not precluded from responding to CLL B cells infected with a gene therapy vector containing the accessory molecule ligand gene.

v. Autologous T Cell Responses to CLL Cells Into Which a Gene Therapy Vector Encoding a Murine Accessory Molecule Ligand Gene Has Been Introduced

T cells isolated from the blood of CLL patients were examined for their ability to respond in vitro to autologous CLL B cells containing a gene therapy vector which encodes the murine accessory molecule, CD40 ligand. T cells were isolated to $>95\%$ purity, and then co-cultured with mitomycin-C-treated autologous leukemia

cells in serum-free AIM-V medium supplemented with exogenous interleukin-2 at 25 U/ml. Modest ^3H -thymidine incorporation ($\leq 10,000$ cpm) was detected in cultures without added stimulator cells, secondary in part to the exogenous IL-2 (Figure 13A, and data not shown). The level of T cell proliferation, however, did not increase in response to uninfected CLL cells (Figure 13A, CLL) or Ad-lacZ-infected CLL cells (Figure 13A, lacZ-CLL). In contrast, CLL cells infected with a gene therapy vector containing the accessory molecule ligand (Figure 13A, CD154-CLL) induced autologous T cells to incorporate significantly more ^3H -thymidine ($17,368 \pm 1,093$ cpm, $n=3$) than any of the control cultures ($P < 0.05$, Bonferroni t-test). Furthermore, the MLTR stimulated with CLL cells infected with a vector encoding an accessory molecule ligand (CD40L) also generated significantly more IFN γ (165 ± 3 ng/ml, $n=3$) than any of the other cultures (Figure 13B) ($P < 0.05$, Bonferroni t-test).

The T cells were harvested after 5 days from the autologous MLTR and assessed for CTL activity against autologous CLL B cells. T cells co-cultured with autologous CD40-ligand-CLL cells developed CTL activity for non-modified CLL B cells, effecting 40.1% lysis ($\pm 2.3\%$) at an E:T ratio of 2:1 (Figure 13C, CD154). However, such T cells did not develop detectable CTL activity for the same target cells in the control reactions, when co-cultured with uninfected or Ad-lacZ-infected CLL cells (Figure 13C).

vi. Specificity of CTL Stimulated by Autologous CD40-Ligand-CLL B Cells for Allogeneic CLL B Cells

Effector cells stimulated with autologous CD40-ligand-CLL were evaluated for their ability to secrete IFN γ or manifest CTL activity against allogeneic CLL B cells (Figure 14). After 5 days of autologous MLTR with

CD154-CLL or lacZ-CLL, T cells were isolated by Ficoll density gradient centrifugation, washed extensively, and then cultured in media for 24 h. Washed T cells were mixed with autologous ("Auto CLL", solid bar) or
5 allogeneic ("Allo-1 CLL" or "Allo-2 CLL", shaded or hatched bars) target CLL B cells. T cells stimulated in the autologous MLTR with CD40-ligand-CLL cells, but not with lacZ-CLL cells, produced significantly more IFN γ in response to secondary culture with non-modified
10 autologous CLL B cells than with allogeneic CLL B cells (Figure 14A) ($P < 0.05$, Bonferroni t-test). Furthermore, T cells stimulated with CD40-ligand-CLL cells, but not with lacZ-CLL cells, were cytotoxic for autologous CLL cells, but not allogeneic CLL cells (Figure 14B).
15 Similar results were obtained with the autologous MLTR-activated T cells of the allogeneic donor, again demonstrating specific cytotoxicity for autologous CLL B cells (data not shown). Finally, W6/32, a mAb to class I major histocompatibility complex (MHC I) antigens
20 could significantly inhibit the cytotoxicity of T cells stimulated with CD40-ligand-CLL cells for autologous CLL B cells (Figure 14C, α HLA-class I) ($P < 0.05$, Bonferroni t-test). Such inhibition was not observed with mAb specific for MHC class II antigen (Figure 14C,
25 α HLA-DP), mAb specific for the Fas-ligand (Figure 14C, α FasL), or an isotype control mAb of irrelevant specificity (Figure 14C, MOPC-21). Collectively, these studies indicate that Ad-CD40-ligand-infected CLL cells can induce an autologous anti-leukemia cellular immune
30 response in vitro, leading to the generation of MHC-class I-restricted CTL specific for autologous non-modified leukemia B cells.

e. Transactivation of Non-Infected Bystander Leukemia B Cells by Ad-CD40L CLL Cells

35 To address whether the changes in tumor marker expression (described in section 1di.) resulted from

intracellular versus intercellular stimulation, the effect of culture density on the induced expression of CD54 and CD80 following infection with adenovirus gene therapy vector encoding the accessory molecule ligand (CD40L, or CD154) was examined. After infection, CLL cells were cultured at standard high density (e.g. 1×10^6 cells/ml) or low density (e.g. 2×10^5 cells/ml) for 3 days at 37° C. Cells plated at high density contained homotypic aggregates, whereas cells plated at low density remained evenly dispersed and without substantial cell-cell contact (data not shown). Despite expressing similar levels of heterologous CD154, CD154-CLL B cells cultured at high density were induced to express higher levels of CD54 and CD80 than CD154-CLL cells cultured at low density (Figure 15A). The stimulation achieved at high density could be inhibited by culturing the cells with a hamster anti-mouse CD154 mAb capable of blocking CD40<->CD154 interactions (Figure 15B, aCD154 Ab). Collectively, these studies indicate that CD154-CLL cells can activate each other in trans and that surface expression of CD154 is necessary for optimal leukemia cell stimulation.

In addition, Ad-CD154-infected, uninfected, Ad-lacZ-infected, or G28-5-stimulated CLL cells were labeled with a green-fluorescence dye to examine whether CD154-CLL could stimulate non-infected bystander leukemia cells. Dye-labeled cells were used as stimulator cells for equal numbers of non-labeled syngeneic CLL B cells. After 2 days' culture, stimulator cells cultured by themselves retained the green-fluorescence dye, allowing such cells to be distinguished from non-labeled CLL cells by flow cytometry. Bystander (green-fluorescence-negative) CD19⁺ CLL B cells were induced to express CD54 (Figure 15C, right histogram) or CD86 (Figure 15D, right histogram) when co-cultured with Ad-CD154-infected leukemia B cells, but not with mock infected CLL cells (Figures 15C

and 15D, left histograms), G28-5-stimulated CLL cells, or Ad-lacZ-infected CLL cells (data not shown). As expected, these bystander (green-fluorescence-negative) CLL cells also were negative for heterologous CD154.

5 f. Treatment of Leukemia with Gene Therapy

Vectors Encoding an Accessory Molecule Ligand

Figure 24 shows an outline for a clinical trial for testing treatment of B cell CLL with adenovirus gene therapy vectors encoding modified CD40 ligand. Leukemia
10 cells harvested by pheresis are infected with replication-defective vectors that encode the modified CD40 ligand. Following expression of this protein, the cells will be administered back to the patient for the purpose of stimulating a host anti-leukemia-cell immune
15 response. This strategy is far superior to one that uses gene therapy to affect expression of only one immune stimulatory molecule on the leukemia cell surface. Indded, this strategy results in the leukemia cells expressing an array of immune-stimulatory
20 accessory molecules and cytokines, as well as a molecule that can affect the same changes in leukemia cells of the patient that were never harvested.

2. Expression of Chimeric Accessory Molecule Ligand Genes

25 The chimeric accessory molecule ligand genes described below are prepared using standard techniques as described herein.

 a. Preparation of Chimeric Accessory Molecule Ligand Genes Utilizing Domains from Two
30 Different Accessory Molecule Genes

The human CD40 ligand gene was isolated from RNA prepared from T cells which had been activated by an anti-CD3 monoclonal antibody using 5' and 3' primers together with well known PCR methods. Chimeric
35 accessory molecule genes of human CD40 ligand and murine

CD40 ligand are constructed from the newly cloned human CD40 ligand gene and mouse CD40 ligand gene described herein as SEQ ID NO: 2. The transmembrane and cytoplasmic domains of human CD40 ligand genes are exchanged with those of the murine CD40 ligand gene and designated H(Ex)-M(Tm-Cy) CD40 ligand. These chimeric accessory molecule ligand genes are produced using the gene conversion technique described as SOEN which has been previously described by Horton, Mol. Biotechnol., 3:93 (1995). A diagram depicting the chimeric accessory molecule ligand genes which are produced is shown in Figure 4. The nucleotide sequences of each of these respective chimeric accessory molecule ligand genes is designated SEQ ID NOS: 3-7 as indicated in the Table below.

Table IIIChimeric Accessory Molecule Ligand Gene SEQ ID NO:

	HuIC/HuTM/MuEX CD40-Ligand	SEQ ID NO: 3
	HuIC/MuTM/HuEX CD40-Ligand	SEQ ID NO: 4
20	HuIC/MuTM/MuEX CD40-Ligand	SEQ ID NO: 5
	MuIC/HuTM/HuEX CD40-Ligand	SEQ ID NO: 6
	MuIC/MuTM/HuEX CD40-Ligand	SEQ ID NO: 7

Adenovirus vectors encoding each of the chimeric accessory molecules shown in Figure 2 are constructed using the methods described in Example 1. Each of these constructs are then transfected into either HeLa cells or CLL cells according to the methods of Example 1.

b. Expression of Chimeric Accessory Molecule Ligands on CLL and HeLa Cells

The expression of each of the chimeric accessory molecule ligand genes constructed above is analyzed by using FACS analysis as specified in Example 1. The appropriate monoclonal antibody immunospecific for the external domain of either human or mouse CD40 ligand is selected and used to determine the level of expression

of the chimeric accessory molecules on the surface of these cells. After appropriate analysis and preparation of appropriate histograms, the expression of chimeric accessory molecules containing at least a portion of the murine CD40 ligand gene is confirmed.

c. Function of Chimeric Accessory Molecule Ligands

CLL cells are infected with various MOI of the mCD40L adenovirus and then cultured in 48 or 24 well tissue culture plates for various times after infection (48, 72, and 96 hours). The CD19⁺ B cells are then analyzed by multiparameter FACS analysis for induction of CD80 and CD54 expression using fluroescein isothiocyanate-conjugated mAb specific for each respective surface antigen as described in Example 1. Increased amounts of CD54 and CD80 are found on cells which have the chimeric accessory molecules containing the domain or domains derived from the mouse CD40 ligand gene.

Further analysis of the cells containing the chimeric accessory molecule genes is carried out according to Example 1(d). The cells containing the chimeric accessory molecule genes which contain the domains derived from the murine CD40 ligand gene are able to stimulate the production of gamma interferon and T cell proliferation.

d. Expression of Chimeric Accessory Molecule Genes Which Contain Proximal Extracellular Domains from Two Different Accessory Molecules from the Same Species

A chimeric accessory molecule ligand gene is prepared which contains the proximal extracellular domain from the human CD70 gene (Domain III) with the remainder of the domains derived from the human CD40 ligand gene. This gene is prepared using standard

biologic techniques as previously described herein.

This chimeric accessory molecule ligand gene has the DNA sequence shown as SEQ ID NO: 19. A different chimeric accessory molecule ligand gene is prepared which

5 contains the proximal extracellular domain from the murine CD40 ligand gene with the remainder of the domains derived from the human CD40 ligand gene. This gene is prepared using standard techniques as previously described herein. This chimeric accessory molecule
10 ligand gene has the DNA sequence shown as SEQ ID NO: 20.

The chimeric accessory molecule genes shown as SEQ ID NOS: 19 and 20 are inserted into the appropriate vectors as described in Example 1 and introduced into human neoplastic cells. The expression of that chimeric
15 accessory molecule gene in the cells is determined as was described in Example 1.

The chimeric accessory molecule encoded by each of these chimeric accessory molecule genes is found on the surface of the human neoplastic cells using the FACS
20 analysis described in Example 1. Increased amounts of CD54 and CD80 are found on the cells containing the chimeric accessory molecule genes using the techniques described in Example 1. The cells containing the chimeric accessory molecule gene are able to stimulate
25 the production of gamma interferon and T cell proliferation as described and assayed according to Example 1.

3. Augmentation of Vaccination Using Vectors Encoding Accessory Molecules

30 The following procedures were used to demonstrate the augmentation of a vaccination protocol using a gene therapy vector encoding an accessory molecule.

a. Augmentation of the Antibody Response in Mice Co-
Injected with an Accessory Molecule Gene
Therapy Vector and placZ

Three different gene therapy constructs were
5 prepared using standard techniques including those
techniques described herein. The first was a control
gene therapy vector, pcDNA3, which did not contain any
gene. The second, placZ, contained the Lac-Z gene which
encoded β -galactosidase (β -gal). The third, p-mCD40L,
10 contained the murine CD40 ligand gene described in
Example 1.

Prior to any immunizations, serum was isolated from
6-8 week old BALB/c-mice to determine the amount of any
initial antibodies to β -galactosidase. Each animal was
15 injected i.m. with 100 micrograms of plasmid DNA per
injection. Four separate injections were given at one
week intervals.

Prior to the third injection, the animals were bled
to monitor the early antibody response to β -gal. One
20 week after the final injection of plasmid DNA, the
animals were bled to monitor the late antibody response
to beta-galactosidase. To test the sensitivity of the
assay, known amounts of anti- β -gal antibodies isolated
from an anti- β -gal antiserum were tested in parallel.

25 Serum dilutions of 1:40, 1:200, or 1:1000 were
tested in an ELISA for anti- β -gal antibodies. For this,
polystyrene microtiter ELISA plates were coated with β -
gal at 10 microgram/ml in phosphate buffered saline.
The plates were washed thrice with blocking buffer
30 containing 1% bovine serum albumin (BSA), 0.2% Tween 20
in borate buffered saline (BBS) (0.1M borate, 0.2M NaCl,
pH 8.2). 50 microliters of diluted serum were added to
separate wells. After at least 1 hour at room
temperature, the plates were washed thrice with blocking
35 buffer and then allowed to react with alkaline
phosphatase-conjugated goat anti-mouse IgG antibody.
One hour later, the plates again were washed four times

with blocking buffer and incubated with 25 ml of TMB peroxidase substrate (Kirkegaard & Perry, Gaithersburg, MD). The absorbance at 405 nm of each well was measured using a microplate reader (Molecular devices, Menlo Park, CA). The higher the O.D. reading, the greater the amount of specific antibody in the sample.

The data for each of two experiments are provided in Tables IV and V which follow on separate sheets. The results are summarized in Tables VI and VII collating the data from the two experiments is provided as well. On the summary page *n* stands for the number of animals in each of the four groups. S.D. stands for standard deviation and Avg. is the average O.D. reading for all the animals in a particular group.

The results of Group 4 demonstrate that the use of a gene therapy vector encoding an accessory molecule ligand (CD40L) enhances the immunization against β -gal encoded by a genetic or gene therapy vector. The average O.D. reading of the 1:40 dilution of the sera from animals of this group is significantly higher than that of groups 1, 2, and 3 ($P < 0.05$, Bonferroni *t* tests, see Table VII).

Data from an additional experiment further reinforce the finding that the gene therapy vector encoding an accessory molecule ligand enhances immunization against β -gal (Figure 16). Here, pCD40L and placZ were co-injected into skeletal muscle, to test for enhancement of the immune response to placZ, a pcDNA3-based vector encoding *E. coli* β -galactosidase. The relative anti- β -gal Ab activities were determined via ELISA. As expected, mice injected with either the non-modified pcDNA3 vector or pCD40L alone did not produce detectable antibodies to β -gal (Figure 16A). Mice were injected with either 100 μ g pcDNA3 (checkered bar), 50 μ g pcDNA3 + 50 μ g pCD40L (lined bar), 50 μ g pcDNA3 + 50 μ g placZ (striped bar), or 50 μ g pCD40L + 50 μ g placZ (solid bar). On the other hand, mice that

received placZ and pCDNA3 developed detectable anti- β -gal antibodies one week after the fourth and final injection, at d28. Mice that received placZ and pCD40L developed higher titers of anti- β -gal antibodies than mice injected with placZ and pCDNA3. Figure 16B, ELISA analyses of serial dilutions of sera collected at d28, shows that mice co-injected with placZ and pCD40L had an eight-fold higher mean titer of anti- β -gal antibodies at d28 than mice treated with placZ + pCDNA3.

10 i. Immunoglobulin Subclass Production
 Stimulated by Accessory Molecule Vector
 Co-Injection

Despite enhancing the titer of the anti- β -gal antibody response, the subclass of anti- β -gal IgG induced by injection of placZ was not altered by the co-injection of pCD40L. IgG_{2a} anti- β -gal antibodies predominated over IgG₁ subclass antibodies in the sera of mice injected with either placZ and pCDNA3 or placZ and pCD40L (Figure 17). Also depicted are the ELISA O.D. measurements of anti- β -gal IgG₁ and anti- β -gal IgG_{2a} present in the pre-immune sera (striped bar) or post-immune sera (solid bar), collected at d28) of each group of mice, injected as indicated on the abscissa. In contrast, BALB/c mice injected with β -gal protein developed predominantly IgG₁ anti- β -gal antibodies, and no detectable IgG_{2a} anti- β -gal antibodies.

 ii. Augmentation of Vaccination by Accessory
 Molecule Vector Requires Co-Injection
 with placZ at the Same Site

30 The adjuvant effect of the pCD40L plasmid on the anti- β -gal antibody response was noted only when it was injected into the same site as placZ (Figure 18). Groups of BALB/c mice (n=4) received intramuscular injections of placZ and pCD40L together at the same site, or as simultaneous separate injections at distal

sites (right and left hind leg quadriceps). A control group received intramuscular injections of placZ and pCDNA3 at the same site. Animals were bled at d28 and the sera tested for anti- β -gal Ab at different dilutions, as indicated on the abscissa. The graph illustrates a representative experiment depicting the mean O.D. at 405 nm of replicate wells of each of the serum samples for each group, at a 1:40, 1:200, or 1:1000 dilution. Animals injected simultaneously with placZ and pCD40L, but at different sites, did not develop detectable anti- β -gal antibodies until d28. Moreover, the anti- β -gal antibody titers of the sera from such animals at d28 were similar to that of mice that received placZ and pCDNA3, and significantly less than that of animals that received placZ and pCD40L together at the same site.

iii. Augmentation of Vaccination When
Accessory Molecule Vector and placZ are
Co-Injected into Dermis

The pCD40L plasmid also enhanced the anti- β -gal antibody response to placZ when injected into the dermis. In the experiment shown in Figure 19, mice received intradermal injections, near the base of the tail, with either 50 μ g pCDNA3 (checkered bar), 25 μ g pCDNA3 + 25 μ g pCD40L (lined bar), 25 μ g pCDNA3 + 25 μ g placZ (striped bar), or 25 μ g pCD40L + 25 μ g placZ (solid bar). Injections, bleeds and ELISA analyses were performed as in Figure 16A. The checkered bar and lined bar groups each consisted of 8 mice while the striped bar and solid bar groups each consisted of 12 mice. The height of each bar represents the mean O.D. of sera at a 1:40 dilution of each group \pm S.E. A statistical analysis of the data indicated that the striped bar and solid bar groups are independent ($P < .05$). As observed with intramuscular injection, mice co-injected with placZ and pCD40L developed detectable serum anti- β -gal

antibodies one week following the second injection (d14), and two weeks earlier than mice injected with placZ and pcDNA3. Moreover, these animals also had an eight-fold higher mean titer of anti- β -gal antibodies than mice of the placZ-injected group at d28. Mice injected with either the non-modified pcDNA3 vector or pCD40L alone did not produce detectable antibodies to β -gal.

10 b. Augmentation of the CTL Response in Mice Co-
 Injected with an Accessory Molecule Gene
 Therapy Vector and placZ

The ability of pCD40L to enhance induction, by placZ, of CTL specific for syngeneic b-gal-expressing target cells was tested. BALB/c mice co-injected with pCD40L and placZ into skeletal muscle (Figure 20A) or dermis (Figure 20B) generated greater numbers of CTL specific for P13.2, a placZ transfected P815 cell line, than mice co-injected with placZ and pcDNA3. At a 5:1 effector:target ratio, the splenocyte effector cells from mice that received intramuscular injections of placZ and pCD40L achieved greater than 20% specific lysis of P13.2. In contrast, when splenocytes of mice that received the control injection with placZ and pcDNA3 were used, a 9-fold greater ratio of effector to target cells was required to achieve this level of specific lysis. Similarly, the splenocyte effector cells from mice that received intradermal injections of placZ and pCD40L killed more than 50% of the P13.2 cells at effector:target ratios of 4:1. To achieve comparable levels of specific lysis required eight-fold higher effector:target ratios using splenocytes from mice that received intradermal injections of placZ and pcDNA3. Nevertheless, the splenocytes of mice co-injected with pCD40L and placZ did not have greater non-specific CTL activity for P815 cells than that of mice that received placZ along with pcDNA3 (Figure 20). As expected, the

splenocytes from mice that received injections of pcDNA3 alone, or pcDNA3 and pCD40L, did not mediate specific lysis of P13.2 or P815 cells.

Table IV

Experiment #1	Injections of plasmid DNA i.m.: 4/3/96; 4/10/96; 4/17/96; 4/24/96											
ELISA for anti-beta galactosidase												
antibodies:												
Group	Animal	Dilution of Pre-Bleed (4/3)			Dilution of Bleed (4/17)			Dilution of Bleed (5/1)				
		1/140	1/200	1/1000	1/140	1/200	1/1000	1/40	1/200	1/1000		
5	pcDNA3 (p-control, 100 mcg)	1	0.09	0.11	0.09	0.06	0.06	0.06	0.11	0.17	0.11	
	(Control vector)	2	0.11	0.09	0.09	0.07	0.07	0.10	0.09	0.08	0.08	
		3	0.12	0.11	0.10	0.09	0.09	0.10	0.12	0.08	0.08	
		4	0.11	0.10	0.10	0.08	0.11	0.07	0.11	0.07	0.08	
	Avg.		0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	
	S.D.		0.01	0.01	0.01	0.02	0.02	0.01	0.04	0.01	0.01	
10	p-lacZ (50 mcg)	5	0.13	0.10	0.10	0.07	0.11	0.06	0.15	0.10	0.08	
	+	6	0.10	0.11	0.10	0.07	0.06	0.06	0.22	0.15	0.14	
	p-Control (50 mcg)	7	0.19	0.10	0.18	0.07	0.07	0.06	0.78	0.29	0.12	
		8	0.10	0.09	0.10	0.08	0.07	0.07	3.04	1.84	0.77	
	Avg.		0.13	0.10	0.12	0.07	0.08	0.06	1.05	0.60	0.28	
	S.D.		0.04	0.01	0.04	0.01	0.02	1.36	0.84	0.33		
	p-lacZ (50 mcg)	27	0.06	0.06	0.06	0.13	0.11	0.08	2.30	1.68	0.72	
	+	18	0.06	0.06	0.06	0.27	0.13	0.10	2.35	0.09	0.28	
	pRCMV-mCD40L (p-mCD40L,	19	0.06	0.06	0.06	0.23	0.19	0.11	2.06	1.09	0.39	
		20	0.06	0.06	0.06	0.23	0.19	0.11	2.06	1.09	0.39	
	Avg.		0.06	0.06	0.06	0.24	0.21	0.21	2.25	1.00	0.47	
	S.D.		0.00	0.00	1.06	0.66	0.24	0.13	0.67	0.19		

Table V

Experiment #2	Injections of plasmid DNA I.m.: 6/5/96; 6/12/96; 6/19/96; 6/26/96										
Dilutions of sera for anti-beta galactosidase antibodies:											
Group	Animal	Dilution of Pre-Blood (6/5)			Dilution of Blood (7/19)			Dilution of Blood (8/3)			
		1/140	1/200	1/1000	1/140	1/200	1/1000	1/40	1/200	1/1000	
5	p-Control (50 mcg)	9	0.02	0.02	0.08	0.04	0.01	0.01	0.04	0.03	0.05
	+	10	0.06	0.02	0.10	0.02	0.02	0.00	0.08	0.09	0.05
	p-mCD40L (50 mcg)	11	0.02	0.02	0.07	0.03	0.01	0.00	0.02	0.02	0.05
		12	0.06	0.03	0.05	0.18	0.04	0.01	0.11	0.04	0.05
	Avg.		0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
	S.D.		0.02	0.01	0.02	0.07	0.01	0.01	0.04	0.03	0.00
	p-lacZ (50 mcg)	5	0.02	0.03	0.02	0.06	0.04	0.04	0.39	0.11	0.03
	+	6	0.03	0.02	0.03	0.14	0.03	0.04	2.85	1.58	0.41
	p-Control (50 mcg)	7	0.56	0.13	0.06	0.29	0.06	0.02	0.22	0.07	0.03
10		8	0.01	0.02	0.05	0.06	0.02	0.02	0.11	0.04	0.05
	Avg.		0.15	0.05	0.04	0.13	0.04	0.03	0.89	0.45	0.13
	S.D.		0.27	0.05	0.02	0.11	0.02	0.01	1.31	0.75	0.19
	p-lacZ (50 mcg)	13	0.23	0.06	0.05	0.28	0.07	0.02	2.37	0.73	0.18
	+	14	0.02	0.02	0.03	0.04	0.02	0.01	3.05	2.23	0.59
	p-mCD40L (50 mcg)	15	0.02	0.02	0.02	0.89	0.21	0.05	2.46	0.96	0.21
		16	0.05	0.04	0.02	0.11	0.04	0.04	2.75	1.39	0.34
	Avg.		0.08	0.04	0.03	0.33	0.08	0.03	2.66	1.33	0.33
	S.D.		0.10	0.02	0.02	0.39	0.09	0.02	0.31	0.67	0.19

Table VI

Summary	Pre-immune @ beta-gal						Early @ beta-gal						Late @ beta-gal					
			1/140		1/200		1/1000		1/140		1/200		1/1000		1/40		1/200	
	Avg.		0.11		0.11		0.11		0.11		0.11		0.11		0.11		0.11	
1) p-Control (n = 4)																		
	Avg.		0.11		0.11		0.11		0.11		0.11		0.11		0.11		0.11	
	S.D.		0.01		0.01		0.01		0.01		0.02		0.02		0.01		0.04	
2) p-mCD40L + p-Control (n = 4)																		
	Avg.		0.04		0.04		0.04		0.04		0.04		0.04		0.04		0.04	
	S.D.		0.02		0.01		0.02		0.07		0.01		0.01		0.04		0.03	
3) p-lacZ + p-Control (n = 8)																		
	Avg.		0.11		0.04		0.04		0.11		0.03		0.03		0.61		0.31	
	S.D.		0.22		0.04		0.01		0.09		0.02		0.02		1.11		0.62	
4) p-lacZ + p-mCD40L (n = 8)																		
	Avg.		0.11		0.04		0.03		0.25		0.06		0.03		2.06		1.04	
	S.D.		0.10		0.02		0.01		0.32		0.07		0.02		0.97		0.69	

88

Anti-beta-galactosidase standard:

67 ng	22ng	7.4ng	2.5ng	.82ng	.27ng
3.01	2.98	2.05	1.10	0.52	0.26
3.14	3.14	2.25	1.20	0.58	0.26

O.D.

Table VII

BONFERRONI t-TESTS

Comparison	Difference of means			t	P<.05
4 vs 2:	2.06 -	0.04 =	2.02	3.782	Yes
4 vs 1:	2.06 -	0.11 =	1.95	3.651	Yes
4 vs 3:	2.06 -	0.61 =	1.45	3.325	Yes
3 vs 2:	0.61 -	0.04 =	0.57	1.067	No
3 vs 1:	0.61 -	0.11 =	0.50	Do not test	
1 vs 2:	0.11 -	0.04 =	0.07	Do not test	

10 Degrees of freedom: 20

ONE WAY ANALYSIS OF VARIANCE

Group	N	Mean	Std Dev	SEM
1	4	0.11	0.01	0.00
2	4	0.04	0.04	0.02
3	8	0.61	1.11	0.39
4	8	2.06	0.97	0.34
5	4	1.51	0.77	0.38
6	4	1.14	0.53	0.26
7	4	0.83	0.43	0.22

20 ONE WAY ANALYSIS OF VARIANCE

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	18.29	6	3.05
Within Groups	18.39	29	0.63
Total	36.69	35	

25

$$F = \frac{s2_bet}{s2_wit} = \frac{MSbet}{Mswit} = \frac{3.05}{0.63} = 4.81 \quad P = 0.002$$

4. Treatment of Neoplasia Using a Gene Therapy Vector Containing an Accessory Molecule Gene or Chimeric Accessory Molecule Gene

a. Treatment of Neoplasia in Mice

5 The treatment of a neoplasia in a mouse model system has been demonstrated using the genes encoding accessory molecule ligands of the present invention. Gene therapy vectors containing an accessory molecule ligand gene (murine CD40 ligand) were prepared as has
10 been previously described in the above examples. These gene therapy vectors were used to introduce that accessory molecule ligand gene into neoplastic cells, Linel cells, from a tumor which originated in BALB/c mice. The accessory molecules were introduced into the
15 neoplastic cells according to the above examples. The expression of the accessory molecule ligand on the surface of these neoplastic cells was confirmed using flow cytometry as has been described in the above examples.

20 The effectiveness of the accessory molecule ligand genes for treating neoplasia was shown as follows. Female BALB/c mice (6-8 weeks old) were injected i.p. with 1.0×10^5 irradiated Linel neoplastic cells. The neoplastic Linel cells are derived from a spontaneous
25 lung adenocarcinoma in a BALB/c mouse. This neoplastic cell has been described by Blieden et al., Int. J. Cancer Supp., 6:82 (1991). Other female BALB/c mice were injected i.p. with 1.0×10^5 irradiated Linel tumor cells that had previously been transduced with the gene
30 therapy vector encoding the accessory molecule ligand gene (murine CD40) as described above.

Each group of mice was allowed to generate an immune response for 10 days. After 10 days each mouse was challenged with 1.0×10^4 live, non-irradiated Linel
35 neoplastic cells. These mice were then monitored for the formation of tumors and then sacrificed when the tumors grew to 2.0 cm because of morbidity. The results

of this monitoring are shown in Figure 7. As can be seen by Figure 7, the mice immunized with the neoplastic cell expressing the accessory molecule ligands of the present invention on the cell surface remained free of tumor throughout the experiment. Mice immunized with the neoplastic cells not having the accessory molecule ligand genes of the present invention succumbed to tumor 50 days after challenge with the neoplastic cells.

Figure 21 demonstrates downmodulation of human CD40L, but not murine CD40L, in lung tumor cell lines that express CD40. Human cell lines HeLa (CD40-negative cervical carcinoma, Figure 21A), A427 (CD40-negative lung carcinoma, Figure 21B), NCI 460 (weakly CD40-positive lung large cell carcinoma, Figure 21C), and SK-Mes-1 (strongly CD40-positive lung squamous cell tumor, Figure 21D) were infected with adenovirus encoding lac-Z (Ad-LacZ), murine CD40L (Ad-mCD40L), and human CD40L (Ad-hCD40L) at an MOI of 0 (Blank), 1, and 10. 48 hours after infection, murine CD40L and human CD40L surface expression was determined. The percentage of cells that express ligand are plotted on the Y-axis. Human and mouse CD40L are expressed at equal levels in CD40-negative cell lines. However, only murine CD40L expression is stable on cell lines that express CD40. In contrast to mCD40L, human CD40L is downmodulated on CD40-positive tumors.

The data graphed in Figure 22A show that CD40 binding induces expression of tumor surface markers. Treating CD40-expressing lung cancer cell lines with α CD40 mAb resulted in enhanced expression of the tumor cell surface markers CD95 (Fas), CD54 (ICAM-1) and class I major histocompatibility antigens (MHC I). NCI 460, a weakly CD40-positive lung large cell carcinoma, was incubated with a CD40-specific monoclonal antibody (thick line), or MOPC21, an isotype control mAb (thin line), on CD32-expressing mouse fibroblasts for 48 hours. Following the 48 hr incubation, the lung tumor

cells were analyzed for CD95, CD54, and MHC-I expression by FACS.

Figure 22B again shows downmodulation of human CD40L by CD40-positive tumor cells. HeLa (CD40-negative), CLL (CD40-positive), and SK-MES-1 (CD40-positive) tumor cells were cocultured for 24 hours with CD3-activated normal donor T cells at a tumor cell:T cell ration of 2.5:1. Following coculture, CD2-expressing T cells were analyzed for CD40L surface expression by FACS. Thin lines represent T cells stained with FITC-labeled isotype control antibody (MOPC21) and thick lines represent activated T cells stained with FITC-labeled α CD40L antibody (α CD154 antibody). The CD40-positive tumor cell lines, SK-MES-1, and CLL, do not express CD40 ligand on their surfaces.

5. Expression of the Human and Mouse Accessory Molecule Ligand, Fas Ligand, in Human Blood Lymphocytes

20 a. Construction of a Genetic Construct and Gene Therapy Vector Containing the Human and Mouse Fas Ligand Gene

Either the human accessory molecule ligand gene (human Fas ligand) or the murine accessory molecule ligand gene (murine Fas ligand) was constructed utilizing the respective human and murine genes. An altered accessory cell molecule, in which a putative MMP-cleavage site was removed, was made and designated Δ FasL-pcDNA3. The nucleotide sequence of Δ FasL-pcDNA3 is listed as SEQ ID NO: 40. Human Fas ligand nucleotides 325 to 342, encoding six amino acids, are missing from Δ FasL. The design of Δ FasL was based on reasoning that Domain III contains sites most accessible to MMPs, and could thus be the target on the molecule for cleavage from the surface of the cell. Sequences of the human Fas ligand gene have been determined and are

listed as SEQ ID NOS: 13 and 30 (Genbank accession U11821). Sequences of mouse Fas ligand genes have been determined and are listed as SEQ ID NOS: 14 (C57BL/6, Genbank accession U10984) and 31 (Balb/c, Genbank accession U58995). The sequence of the rat Fas ligand gene has been determined and is listed as SEQ ID NO: 25 (Genbank accession U03470). Chimeric constructs are made, as described in Example 2 for CD40 ligand chimeric constructs, in which Domain III of human Fas ligand is replaced with Domains of other proteins, particularly proteins of the TNF family. Chimeric constructs include, but are not limited to, human Fas ligand with Domain III replaced by Domain III of murine Fas ligand (chimeric sequence listed as SEQ ID NO: 37, sequence line-up shown in Figure 37), or replaced by Domain III of human CD70 (chimeric sequence listed as SEQ ID NO: 38, sequence line-up shown in Figure 38), or replaced with Domain I of human CD70 (chimeric sequence listed as SEQ ID NO: 39, sequence line-up shown in Figure 39). Chimeric constructs in which multiple domains, for example, two copies of human CD70 Domain III, are inserted into human Fas ligand in place of Domain III, are also made using methods described in Example I. Chimeric constructs in which synthetic sequences are used to replace Domain III of human Fas ligand are also made.

i. Human Fas Ligand Cloning

The cDNA encoding human Fas-ligand was subcloned in the eukaryotic expression vector pcDNA3. Normal donor blood lymphocytes were activated for 4 hours with 1 ng/ml PMA plus 0.5 uM ionomycin. Total RNA was isolated with the Qiagen Rneasy kit. cDNA was then synthesized from poly-A RNA with oligo-dT primers using the Gibco-BRL Superscript cDNA synthesis kit. The gene encoding human Fas-ligand was then PCR amplified with the Fas-ligand-specific primers (sense primer, SEQ ID NO: 32,

antisense primer, SEQ ID NO: 33). The Fas-ligand PCR product was then subcloned into pcDNA3 using standard molecular biology techniques. RT-PCR products, subcloned into pcDNA3, are designated hFasL-pcDNA3.

5 ii. Murine Fas Ligand Cloning

The murine Fas-ligand genes from Balb/c and C57/BL6 strains of mice were also amplified following activation of mouse splenocytes with PMA plus ionomycin as described above, and amplified from poly-A synthesized
10 cDNA as described above (sense primer, SEQ ID NO: 34, antisense primer, SEQ ID NO: 35). These genes were subcloned in the pTARGET expression vector (Promega, Madison, WI). RT-PCR products, subcloned into pcDNA3, are designated mFasL-pcDNA3.

15 iii. Adenovirus Vector Construction

For construction of adenovirus vectors encoding human Fas-ligand, murine Fas-ligand or Δ Fas-ligand, the cloned cDNA insert is subcloned into the plasmid pRc/RSV (Invitrogen, San Diego, CA) at the HindIII-XbaI site. A
20 BglII-XhoI fragment with the RSV promoter-enhancer and the bovine growth hormone poly-A signal sequence was subcloned into the BamHI-XhoI site of plasmid MCS(SK) pXCX2. The plasmid MCS(SK)pXCX2 is a modification of the plasmid pXCX2, in which the pBluescript polylinker
25 sequence was cloned into the E1 region. The resulting plasmid then is co-transfected along with pJM17 into 293 cells using the calcium phosphate method. Isolated plaques of adenovirus vectors are picked and expanded by infecting 293 cells. High titer adenovirus preparations
30 are obtained, as described above which uses a cesium chloride gradient for concentrating virus particles via a step gradient, with the densities of 1.45g/cm³ and 1.20g/cm³, in which samples are centrifuged for 2 hours in an SW41 rotor (Beckman, Brea, CA) at 25,000 rpm at 4°
35 C. The virus band is desalted using a Sephadex G-25 DNA

grade column (Pharmacia, Piscataway, NJ), and the isolated virus is stored at -70°C in phosphate-buffered saline with 10% glycerol. The titer of the virus is determined by infecting permissive 293 cells at various dilutions and counting the number of plaques. Titers typically range from 10^{10} to 10^{12} plaque forming units/ml. The adenovirus constructs are designated Ad-hFasL, Ad-mFasL and Ad- Δ FasL.

10 b. Introduction of the Murine and Human Fas Ligand Genes into Human Cells

The constructs hFasL-pcDNA3, mFasL-pcDNA3 and Δ FasL-pcDNA3 are transfected into 293 via electroporation. The transfected cells are selected in medium containing G418. Fas-ligand transfectants are screened for expression of the transgene using anti-Fas-ligand antibody and flow cytometry. The methods used are similar to those described for transfection of CD40L into CLL cells.

For FasL-adenovirus infection, 10^6 freshly thawed and washed CLL cells or HeLa cells are suspended in 0.5 to 1 mL of culture medium for culture at 37°C in a 5% CO_2 -in-air incubator. Adenovirus are added to the cells at varying multiplicity of infection (MOI), and the infected cells are cultured for 48 hours, unless otherwise stated, before being analyzed for transgene expression.

 c. Expression of the Fas Ligand Genes in Human Cells

Mice with the lymphoproliferative or generalized lymphoproliferative disorder are unable to delete activated self-reactive cells outside of the thymus. This is related to the fact that, in these mice, interactions between the Fas receptor and an accessory molecule ligand, Fas ligand, are defective. These animals develop numerous disorders including lymphadenopathy, splenomegaly, nephritis, and systemic

autoimmune pathology which resembles that seen in patients with systemic lupus erythematosus or rheumatoid arthritis (RA). It is conceivable that the normal interactions between the Fas receptor and the accessory molecule ligand that are responsible for clearance of activated lymphocytes from joints may be impaired in RA patients.

RA synovial lymphocytes express the Fas receptor at a higher proportion than that of matched RA blood lymphocytes to matched normal donor blood lymphocytes. On the other hand, RA synovial lymphocytes express little or no accessory molecule ligand. Since the RA synovial lymphocytes are sensitive to Fas-induced apoptosis, it is feasible that local expression of Fas ligand in the RA joint could serve to eliminate the synovial mononuclear cells that potentially mediate RA autoimmune pathology.

Figure 23 shows that Fas-ligand expression in lymphocytes is inhibited by exposure to RA synovial fluid. Normal donor blood T cells were activated for 5 hours with 1 ng/ml PMA plus 0.5 μ M ionomycin. Cells were incubated in the presence of rheumatoid arthritis blood plasma (circles), RA synovial fluid (diamonds), or neither (squares). In addition, cells were incubated with increasing concentrations of the MMP inhibitor BB94. Following activation, cells were analyzed for Fas-ligand surface expression by FACS. The percentage of cells expressing Fas ligand are plotted in Figure 23. This experiment demonstrates that there is a factor(s) present in RA synovial fluid and serum that prevents surface expression of Fas-ligand.

d. Function of Human, Murine and Chimeric Accessory Molecule Ligand, Fas Ligand

To determine the capacity of the Δ FasL constructs, the above-mentioned transfected cells are mixed with the Fas-ligand sensitive human T cell line, JURKAT. Following 4 hours coculture, the nonadherent JURKAT cells

are collected and evaluated for apoptosis. The fluorescent compound 3,3' dihexyloxacarbocyanine iodide (DiOC₆) is used to evaluate for apoptosis using a modification of a previously described protocol. For this, the cells are washed once at room temperature in phosphate buffered saline (PBS, pH 7.2). Cells are placed into separate wells of a 96 well U-bottom plastic microtiter plate at 10^5 - 5×10^5 cells/well in 50 ml total volume. If indicated, saturating amounts of PE-conjugated antibodies are added followed by addition of DiOC₆ and propidium iodide (PI). DiOC₆ and PI are used at 40 nM and 10 ng/ml final concentrations, respectively. The cells are then incubated 15 minutes in a 37°C, 5% CO₂ tissue culture incubator. The stained cells are then washed twice in ice cold PBS and ultimately suspended in 200 ml SM and analyzed by FACS. Dead cells and debris with characteristic forward and light scatter profiles and PI staining are excluded from analysis.

The ability of cells expressing Δ FasL-pcDNA3 to direct Fas-mediated apoptosis of cells expressing CD95 is compared with that of cells expressing FasL-pcDNA3. Relative stability of the protein products encoded by Δ FasL-pcDNA3 or FasL-pcDNA3 pre- and post- culture with RA synovial fluid, and with or without the metallo-proteinase inhibitors, are assessed via flow cytometry of cells expressing either ligand.

6. Treatment of Arthritis with Gene Therapy Vectors Encoding an Accessory Molecule Ligand, Fas Ligand

The heterologous Fas-ligand constructs, made as described above, that show the highest stability of expression in combination with the greatest ability to mediate Fas-induced apoptosis, are used in gene therapy for RA. Potential therapeutic constructs are tested in well-characterized mouse models of arthritis to assess efficacy and function *in vivo*.

a. Gene Therapy Treatment of Arthritis in Micei. Mouse Models for Arthritis

One mouse arthritis model is collagen-induced arthritis. It is known that injecting DBA/1 mice with
5 type II collagen in complete Freund's adjuvant (CFA)
induces an arthritis with synovitis and erosions that
histologically resemble RA. For our studies, male DBA/I
mice are immunized with bovine type II collagen in
complete Freund's adjuvant on day 0 and boosted
10 intraperitoneally (i.p.) on day 21. On day 28, animals are
given an additional i.p. injection with
lipopolysaccharide (LPS) and/or the same type collagen,
or an injection of acetic acid alone. Swelling and/or
redness of a fore or hind paw in animals immunized with
15 collagen typically is detected the third or fourth week
following the second injection. The vertebrae are only
rarely affected, and then only weeks after the initial
peripheral joint swelling. Affected joints display
initial histologic changes of synovial edema, followed by
20 synovial hyperplasia.

Another animal model, recently described by
Kouskoff, V. et al., in Cell 87:811-822 (1997) was
generated fortuitously, by crossing a T cell receptor
(TCR) transgenic mouse line with the non-obese-diabetic
25 (NOD) strain to produce the KRN x NOD mouse model of RA.
The offspring of such a mating universally develop a
joint disease that is highly similar to that of patients
with RA. Moreover, the disease in these animals has an
early and reproducible time of onset and a highly
30 reproducible course. The arthritis apparently is induced
by chance recognition of an NOD-derived major
histocompatibility complex (MHC) class II molecule by the
transgenic TCR, leading to breakdown in the general
mechanisms of self-tolerance and systemic self-
35 reactivity.

ii. Relief of Arthritis Symptoms in Mice
Treated with a Gene Therapy Vector
Encoding an Accessory Molecule Ligand

We have adapted and modified a protocol originally
5 described by Sawchuk and colleagues for micro-injecting
adenovirus vectors into mouse joints. Using this
procedure we can reproducibly inject a 5 μ l volume into
the articular space of the mouse knee. In this
procedure, the mice are anesthetized with metofane. A
10 small incision of approximately 2-3 mm is made with a #11
scalpel blade in the skin over the lateral aspect of the
knee to visualize the patello-tibial ligament. We can
inject up to 5 μ l of fluid using a micro-100 μ l-Hamilton
syringe and a 30-gauge needle. After the injection, the
15 knee incision is closed with Nexabond (Veterinary
Products Laboratory). Our adenovirus titers typically
exceed 10^{10} plaque forming units (pfu) per ml, making it
possible to deliver at least 5×10^8 pfu of virus in 5 ml
into the knee joints, as outlined above. Control animals
20 are injected with control Ad-lacZ vector, a replication-
defective adenovirus vector lacking a transgene, or with
the buffer used to suspend the virus (10 mM Tris, 1 mM
MgCl₂, 10% glycerol).

In another method, splenocytes will be harvested
25 from mice that are syngeneic to the host animal intended
for adoptive transfer of transduced cells. Cell
proliferation will be induced with exogenous IL-12 (100
units/ml) for 48 h. Cells are counted and then re-plated
at densities of 5×10^5 or 1×10^6 cells per ml in a 12-
30 well dish with 1 ml complete culture medium per well.
Virus and ConA are added together at the time of re-
plating in the presence of polybrene (8 μ g/ml). The
medium is changed 24 hours after infection with complete
medium containing 100 units of recombinant IL-2 per ml.
35 Aliquots of the transduced cells are examined, for Fas-
ligand expression, at 48 hours after infection via flow
cytometry.

Animals will receive standardized numbers of cytokine-producing cells or control mock-transfected cells intraperitoneally. Concentrated cell suspensions are injected directly into the mouse synovium, as described in section 4A above. In parallel, aliquots of the transferred cell populations are maintained in tissue culture supplemented with exogenous IL-2.

Mice are monitored in a blinded fashion for signs of arthritis. The date of disease onset is recorded and clinical severity of each joint or group of joints (toes, tarsus, ankle, wrist, knee) are graded as follows: 0 (normal), 1 (erythema), 2 (swelling), 3 (deformity), 4 (necrosis). The scores are summed to yield the arthritic score. The severity of arthritis is expressed both as the mean score observed on a given day, and as the mean of the maximal arthritic score reached by each mouse during the clinical course of the disease. At the time of death, hind paws are dissected free and processed for histologic examination or for RT-PCR. The histologic severity of the arthritis is scored on a scale of 0-3 for synovial proliferation and inflammatory cell infiltration, where a score of 0 = normal and 3 = severe.

For mice receiving intra-synovial injection of control or test adenovirus vector, the level of arthritis observed between contralateral sites is compared. In addition, the overall joint score minus that of the injected joint for the entire animal is compared with that observed in the joint injected with the control or test adenovirus vector.

Local administration of Fas-ligand adenovirus expression vectors will result in clearance of activated cells, as assessed by measuring the relative levels of CD80 mRNA by quantitative RT-PCR. This treatment also will lead to an enhanced level. Also, whether such level of apoptosis identified in affected mouse synovial tissue is assessed by the TUNEL assay ("Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP Nick End

Labeling"). TUNEL is performed by immersing the sections in TdT buffer (30 mM Tris-HCl, pH 7.2, 140 nM sodium cacodylate, 1 mM cobalt chloride), and then adding TdT (GIBCO BRL, Grand Island, NY) and biotinylated dUTP (Boehringer Mannheim, Indianapolis, IN). The reaction is terminated by immersing the sections in TB buffer (300 mM sodium chloride, 30 mM sodium citrate). Subsequently, the samples are treated with peroxidase-labeled streptavidin and then visualized using the VECTASTAIN ABC kit (Vector Laboratories Inc., Burlingame, CA). For immunohistochemistry, the sections are blocked with 4% skim milk for 30 minutes at room temperature, then incubated with biotinylated mAbs specific for mouse CD3, B220, CD80, or CD95 (Fas). These antibodies are available from Pharmingen (San Diego, CA).

b. Treatment of Rheumatoid Arthritis Patients with a Gene Therapy Vector Encoding an Accessory Molecule Ligand, Fas Ligand

Candidate Fas-ligand constructs identified as having potential therapeutic benefit are used in human protocols to treat RA. Human protocols encompass either *in vivo* or *ex vivo* methods to deliver the Fas-ligand constructs. Furthermore, the Fas-ligand constructs are potentially delivered by either viral or non-viral methods. Outlines of therapeutic strategies are described below.

An *ex vivo* therapy is similar to a protocol described for intra-articular transplantation of autologous synoviocytes retrovirally transduced to synthesize interleukin-1 receptor antagonist (Evan, Christopher et. al., Clinical Trial to Assess the Safety, Feasibility, and Efficacy of Transferring a Potentially Anti-Arthritic Cytokine Gene to Human Joints with Rheumatoid arthritis, Human Gene Therapy, Vol. 7, 1261-1280). In this procedure, after clinical diagnosis of RA, the synovium is harvested during total joint replacement. The synoviocytes re-isolated and expanded,

then transduced or transfected with heterologous Fas-ligand into synoviocytes (via retrovirus, adenovirus, naked DNA, etc.). The gene-modified synoviocytes are then reinjected into the patient, who is monitored and
5 tested for amelioration of RA-associated symptoms, and for expression and function of the Fas-ligand in modified synoviocytes.

In another ex vivo protocol, an allogeneic immortalized cell line that stably expresses the
10 heterologous Fas-ligand is administered to the RA patient. In this protocol, a stable immortalized cell line expressing Fas-ligand (introduced by transfection of the gene into the cell by nonviral methods, such as electroporation), or by viral transduction of the gene
15 into the cell) is constructed. The modified cell line is injected into the patient, who is monitored and tested for amelioration of RA associated symptoms, and for expression and function of the hFas-ligand in modified synoviocytes.

20 An in vivo based therapy will is similar in concept to the amelioration of collagen-induced-arthritis using a murine Fas-ligand adenovirus gene therapy vector, described in Zhang, et al., J. Clin. Invest. 100:1951-1957 (1997). In our use of such an approach, delivery of
25 the hFas-ligand construct or chimeric Δ fasL directly to the joints of RA patients is performed using either viral or non-viral methods. In this procedure, the Fas-ligand construct (e.g. hFas-ligand adenovirus) is directly injected into the synovium. Patients are monitored and
30 tested for amelioration of RA-associated symptoms as well as biological testing for expression and function of the hFas-ligand in modified synoviocytes.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Kipps, Thomas J.
Sharma, Sanjai
Cantwell, Mark
- (ii) TITLE OF INVENTION: NOVEL EXPRESSION VECTORS
CONTAINING ACCESSORY
MOLECULE LIGAND GENES AND
THEIR USE FOR IMMUNOMODULA-
TION AND TREATMENT OF
MALIGNANCIES AND AUTOIMMUNE
DISEASE
- (iii) NUMBER OF SEQUENCES: 35
- (iv) CORRESPONDENCE ADDRESS:
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Suite 4700
(C) CITY: Los Angeles
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(E) COUNTRY: U.S.A.
(F) ZIP: 90071-2066
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5" Diskette,
1.44 Mb storage
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: IBM P.C. DOS 5.0
(D) SOFTWARE: FastSeq Version 2.0
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: To Be Assigned
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 60/132145
(B) FILING DATE: 12/9/96

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Guise, Jeffrey W.
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 (C) REFERENCE/DOCKET NUMBER: 231/003

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 786 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 783 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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AACAAAGAAG	AGAAAAAAGA	AAACAGCTTT	GAAATGCAAA	GAGGTGATGA	GGATCCTCAA	360
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ACGGTTAAAA	GAGAAGGACT	CTATTATGTC	TACACTCAAG	TCACCTTCTG	CTCTAATCGG	540
GAGCCTTCGA	GTCAACGCCC	ATTTCATCGTC	GGCCTCTGGC	TGAAGCCCAG	CATTGGATCT	600

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GAGAGAATCT	TACTCAAGGC	GGCAAATACC	CACAGTTCCT	CCCAGCTTTG	CGAGCAGCAG	660
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(i) SEQUENCE CHARACTERISTICS:

(A)	LENGTH:	783 base pairs
(B)	TYPE:	nucleic acid
(C)	STRANDEDNESS:	single
(D)	TOPOLOGY:	linear

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(i) SEQUENCE CHARACTERISTICS:

(A)	LENGTH:	786 base pairs
(B)	TYPE:	nucleic acid
(C)	STRANDEDNESS:	single
(D)	TOPOLOGY:	linear

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CTCTGA						786

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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 783 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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TCTGTTCACT	TGGGCGGAGT	GTTTGAATTA	CAAGCTGGTG	CTTCTGTGTT	TGTCAACGTG	720
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TGA						783

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 786 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAGTC	CCCCGGTAGA	600
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CAATCCATT	ACTTGGGAGG	AGTATTTGAA	TTGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	720
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(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 786 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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GAAGATTTTG	TATTCATGAA	AACGATACAG	AGATGCAACA	CAGGAGAAAG	ATCCTTATCC	240
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AACAAAGAGG	AGACGAAGAA	AGAAAACAGC	TTTGAAATGC	AAAAAGGTGA	TCAGAAATCCT	360
CAAATTGCGG	CACATGTCAT	AAGTGAGGCC	AGCAGTAAAA	CAACATCTGT	GTTACAGTGG	420
GCTGAAAAAG	GATACTACAC	CATGAGCAAC	AACCTGGTAA	CCCTGGAAAA	TGGGAAACAG	480
CTGACCGTTA	AAAGACAAGG	ACTCTATTAT	ATCTATGCCC	AAGTCACCTT	CTGTTCCAAT	540
CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAGTC	CCCCGGTAGA	600
TTCGAGAGAA	TCTTACTCAG	AGCTGCAAAAT	ACCCACAGTT	CCGCCAAACC	TTGCGGGCAA	660
CAATCCATTC	ACTTGGGAGG	AGTATTTGAA	TGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	720
GTGACTGATC	CAAGCCAAGT	GAGCCATGGC	ACTGGCTTCA	CGTCCTTTGG	CTTACTCAAA	780
CTCTGA						786

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 864 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AACTCTAACG	CAGCATGATC	GAAACATACA	GTCAACCTTC	TCCCCGCTCC	GTGGCCACTG	60
GACCACCTGT	CAGTATGAAA	ATTTTATGT	ATTTACTTAC	AGTTTTTCTT	ATCACCCAGA	120
TGATTGGGTC	AGCGCTTTTT	GCTGTGTATC	TTCACAGACG	ATTGGACAAG	ATAGAAGACG	180
AAAGGAATCT	TCATGAAGAT	TTTGTGTTCA	TGAAAACGAT	ACAGAGATGC	AATAAAGGAG	240
AGGGGTCCTT	ATCCTTACTG	AACTGTGAGG	AAATTAGAAG	CCGGTTTGAA	GACTTGGTCA	300
AGGATATAAT	GCAAAACAAA	GAAGTAAAGA	AGAAAGAAAA	AAACTTTGAA	ATGCACAAGG	360
GTGATCAGGA	GCCTCAGATA	GCGGCACATG	TCATCAGTGA	GGCCAGTAGT	AAAACAACCT	420
CTGTTCTCCA	GTGGGCCCCC	AAAGGATACT	ACACCCTAAG	CAACAACCTG	GTAACCTCG	480
AAAACGGGAA	ACAGCTGGCC	GTGAAAAGAC	AAGGATTCTA	TTACATCTAC	ACCCAAGTCA	540
CCTTCTGTTT	CAATCGGGAA	ACTTTGAGTC	AAGCTCCATT	TATAGCCAGC	CTCTGCCTGA	600
AGTCCCAAG	TGGATCAGAG	AGAATCTTAC	TGAGAGCTGC	AAACACCCAC	AGTTCTTCCA	660
AACCATGCGG	GCAGCAATCC	ATTCACTTAG	GAGGAGTCTT	TGAATTGCAA	TGCGGTGCTT	720
CGGTGTTTGT	CAATGTGACT	GATCCAAGTC	AAGTGAGCCA	CGGGACGGGC	TTCACATCAT	780
TTGGCTTACT	CAAATCTGTA	ACGGTGTAAG	CCAGCAGGCT	GCGGCTGGGC	TGATGCTGGT	840
GGTCTTCACA	ATCCAGGAAA	GCAG				864

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3634 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAATTCGGGG	TGATTTCACT	CCCGGCTGTC	CAGGCTTGTC	CTGCTACCCC	ACCCAGCCTT	60
TCCTGAGGCC	TCAAGCCTGC	CACCAAGCCC	CCAGCTCCTT	CTCCCCGCAG	GACCCAAACA	120
CAGGCCTCAG	GACTCAACAC	AGCTTTTCCC	TCCAACCCGT	TTTCTCTCCC	TCAACGGACT	180
CAGCTTTCTG	AAGCCCCCTC	CAGTTCTAGT	TCTATCTTTT	TCCTGCATCC	TGTCTGGAAG	240
TTAGAAGGAA	ACAGACCACA	GACCTGGTCC	CCAAAAGAAA	TGGAGGCAAT	AGGTTTGGAG	300
GGGCATGGGG	ACGGGGTTCA	GCCTCCAGGG	TCCTACACAC	AAATCAGTCA	GTGGCCACAG	360
AGACCCCCCT	CGGAATCGGA	GCAGGGAGGA	TGGGGAGTGT	GAGGGGTATC	CTTGATGCTT	420
TGTGTGCCCT	GACATTTCCA	ATCCCCGCC	CCCGGATGGA	GAAGAAACCG	AGACAGAAGG	480
TGCAGGGCCC	ACTACCGCTT	CCTCCAGATG	AGCTCATGGG	TTTCTCCACC	AAGGAAGTTT	540

TCCGCTGGTT	GAATGATTCT	TTCCCCGCCC	TCCTCTCGCC	CCAGGGACAT	ATAAAGGCAG	600
TTGTTGGCAC	ACCCAGCCAG	CAGACGCTCC	CTCAGCAAGG	ACAGCAGAGG	ACCAGCTAAG	660
AGGGAGAGAA	GCAACTACAG	ACCCCCCTCG	AAAACAACCC	TCAGACGCCA	CATCCCCTGA	720
CAAGCTGCCA	GGCAGGTTCT	CTTCCTCTCA	CATACTGACC	CACGGCTTCA	CCCTCTCTCC	780
CCTGGAAAGG	ACACCATGAG	CACTGAAAGC	ATGATCCGGG	ACGTGGAGCT	GGCCGAGGAG	840
GGGCTCCCCA	AGAAGACAGG	GGGGCCCCAG	GGCTCCAGGC	GGTGCTTGTT	CCTCAGCCTC	900
TTCTCCTTCC	TGATCGTGCC	AGGCGCCACC	ACGCTCTTCT	GCCTGCTGCA	CTTTGGAGTG	960
ATCGGCCCCC	AGAGGGAAGA	GGTGAGTGCC	TGGCCAGCCT	TCATCCACTC	TCCCACCCAA	1020
GGGGAAATGA	GAGACGCAAG	AGAGGGAGAG	AGATGGGATG	GGTGAAGAT	GTGCGCTGAT	1080
AGGGAGGGAT	GAGAGAGAAA	AAAACATGGA	GAAAGACGGG	GATGCAGAAA	GAGATGTGGC	1140
AAGAGATGGG	GAAGAGAGAG	AGAGAAAGAT	GGAGAGACAG	GATGTCTGGC	ACATGGAAGG	1200
TGCTCACTAA	GTGTGTATGG	AGTGAATGAA	TGAATGAATG	AATGAACAAG	CAGATATATA	1260
AATAAGATAT	GGAGACAGAT	GTGGGGTGTG	AGAAGAGAGA	TGGGGGAAGA	AACAAGTGAT	1320
ATGAATAAAG	ATGTTGAGAC	AGAAAGAGCG	GGAAATATGA	CAGCTAAGGA	GAGAGATGGG	1380
GGAGATAAGG	AGAGAAGAAG	ATAGGGTGTC	TGGCACACAG	AAGACACTCA	GGGAAAGAGC	1440
TGTTGAATGC	TGGAAGGTGA	ATACACAGAT	GAATGGAGAG	AGAAAACCAG	ACACCTCAGG	1500
GCTAAGAGCG	CAGGCCAGAC	AGGCAGCCAG	CTGTTCTCTC	TTTAAGGGTG	ACTCCCTCGA	1560
TGTTAACCAT	TCTCCTTCTC	CCCAACAGTT	CCCCAGGGAC	CTCTCTCTAA	TCAGCCCTCT	1620
GGCCCAGGCA	GTCACTAAGT	GTCTCCAAAC	CTCTTCTCTA	ATTCTGGGTT	TGGGTTTGGG	1680
GGTAGGGTTA	GTACCGGTAT	GGAAGCAGTG	GGGAAAATTT	AAAGTTTGGG	TCTTGGGGGA	1740
GGATGGATGG	AGGTGAAAGT	AGGGGGGTAT	TTTCTAGGAA	GTTTAAAGGGT	CTCAGCTTTT	1800
TCTTTTCTCT	CTCCTCTTCA	GGATCATCTT	CTCGAACCCC	GAGTGACAAG	CCTGTAGCCC	1860
ATGTTGTAGG	TAAGAGCTCT	GAGGATGTGT	CTTGGAACTT	GGAGGGCTAG	GATTTGGGGA	1920
TTGAAGCCCG	GCTGATGGTA	GGCAGAACTT	GGAGACAATG	TGAGAAGGAC	TCGCTGAGCT	1980
CAAGGGAAGG	GTGGAGGAAC	AGCACAGGCC	TTAGTGGGAT	ACTCAGAACG	TCATGGCCAG	2040
GTGGGATGCG	TGTTGACAGA	CAGAGAGGAC	AGGAACCGGA	TGTGGGGTGG	GCAGAGCTCG	2100
AGGGCCAGGA	TGTGGAGAGT	GAACCGACAT	GGCCACACTG	ACTCTCCTCT	CCCTCTCTCC	2160
CCCTCTCCAG	CAAAACCTCA	AGCTGAGGGG	CAGCTCCAGT	GGCTGAACCG	CCGGGCCAAT	2220
GCCCTCCTGG	CCAAATGGCGT	GGAGCTGAGA	GATAACCAGC	TGGTGGTGCC	ATCAGAGGGC	2280
CTGTACCTCA	TCTACTCCCA	GGTCTCTCTC	AAGGGCCAAG	GCTGCCCTCT	CACCCATGTG	2340
CTCTCTACCC	ACACCATCAG	CCGCATCGCC	GTCTCTTACC	AGACCAAGGT	CAACCTCTCT	2400
TCTGCCATCA	AGAGCCCCTG	CCAGAGGGAG	ACCCAGAGG	GGGCTGAGGC	CAAGCCCTGG	2460
TATGAGCCCA	TCTATCTGGG	AGGGGTCTTC	CAGCTGGAGA	AGGGTGACCG	ACTCAGCGCT	2520
GAGATCAATC	GGCCCGACTA	TCTCGACTTT	GCGGAGTCTG	GGCAGGTCTA	CTTTGGGATC	2580
ATTGCCCTGT	GAGGAGGACG	AACATCCAAC	CTTCCCAAAC	GCCTCCCCTG	CCCCAATCCC	2640
TTTATTACCC	CCTCCTTCAG	ACACCCTCAA	CCTCTTCTGG	CTCAAAAAGA	GAATTGGGGG	2700
CTTAGGGTCG	GAACCCAAGC	TTAGAATCTT	AAGCAACAAG	ACCACCACTT	CGAAACCTGG	2760
GATTCAAGAA	TGTGTGGCCT	GCACAGTGAA	GTGCTGGCAA	CCACTAAGAA	TTCAAACTGG	2820
GGCCTCCAGA	ACTCACTGGG	GCCTACAGCT	TTGATCCCTG	ACATCTGGAA	TCTGGAGACC	2880
AGGGAGCCTT	TGGTCTGGGC	CAGAATGCTG	CAGGACTTGA	GAAGACCTCA	CCTAGAAATT	2940
GACACAAGTG	GACCTTAGGC	CTTCTCTCT	CCAGATGTTT	CCAGACTTCC	TTGAGACACG	3000
GAGCCCAGCC	CTCCCCATGG	AGCCAGCTCC	CTCTATTTAT	GTTTGCACTT	GTGATTATTT	3060
ATTATTTATT	TATTATTTAT	TTATTTACAG	ATGAATGTAT	TTATTTGGGA	GACCGGGGTA	3120
TCCTGGGGGA	CCCAATGTAG	GAGCTGCCTT	GGCTCAGACA	TGTTTCCGT	GAAAACGGAG	3180
CTGAACAATA	GGCTGTTCCC	ATGTAGCCCC	CTGGCCTCTG	TGCCCTCTTT	TGATTATGTT	3240
TTTAAATAA	TTTATCTGAT	TAAGTTGTCT	AAACAATGCT	GATTTGGTGA	CCAACCTGCA	3300
CTCATGTCTG	AGCCTCTGCT	CCCCAGGGGA	GTTGTGTCTG	TAATCGCCCT	ACTATTCACT	3360
GGCGAGAAAT	AAAGTTTGCT	TAGAAAAGAA	ACATGGTCTC	CTTCTTGGAA	TTAATTCTGC	3420
ATCTGCCTCT	TCTTGTGGGT	GGGAAGAAGC	TCCTTAAGTC	CTCTCTCCAC	AGGCTTTAAG	3480
ATCCCTCGGA	CCCATGCCA	TCCTTAGACT	CCTAGGGCCC	TGGAGACCCT	ACATAAACAA	3540
AGCCCAACAG	AATATTCCCC	ATCCCCCAGG	AAACAAGAGC	CTGAACCTAA	TTACCTCTCC	3600
CTCAGGGCAT	GGGAATTTCC	AACCTCTGGA	ATTCT			3634

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GAGACAGAGT	CTTGCTCTGT	CCCCCAGGCT	GGAATACAGT	GGTGCGATCT	TGACTCACTG	60
CAGCCTCCGC	CTCCAGGTT	CAAATAATTC	TCCAGCCTCA	GCCTCCCGAG	TAGCTGGGAC	120
TGCAGATGCG	CACCAGCAG	CCTGGCTAAT	TTTTGTATTT	ATTATAGAGA	TGGGGTTTCA	180
CCATGTTGGC	CAGCTGGTCT	CAAACCTCTG	ACCTCAAGTA	ATCCGCCCCAC	CTCAGACTCC	240
CAAAGTGCCA	GGATTACAGG	TGTGAGCCAC	TGCACCAGGC	CTGGAACAAT	TTTAAATAA	300

TGTATTGGCT	CTGCAAATGC	AGCTTCAGAA	CAAGTCCCTT	AGCTGTCCCC	ACCCACCCT	360
AAGTCAACCAC	CCTTAAGCCT	CACCCATGTG	GAATTCTGAA	ACTTCCTTTG	TAGAAAACCTT	420
TGGAAGGTGT	CTGCCACATT	GATCCTGGAA	TGTGTGTTA	TTTGGGGTTA	TATAAATCTG	480
TTCTGTGGAA	GCCACCTGAA	GTCAGGAAGA	GATGGAGGGC	ATCCTTCAGG	AGTGAGATGA	540
GACCTCATCA	TACTTGACTG	TCCAGCATCA	TCTCTGAGTA	AGGGGACCAA	AAAATTTATC	600
TTCCAAACTA	GGACACTTTC	AAGAGTGGAA	GGGGGATCCA	TTAATATTTT	CACCTGGACA	660
AGAGGCAAAAC	ACCAGAATGT	CCCCGATGAA	GGGGATATAT	AATGGACCTT	CTTGATGTGA	720
AACCTGCCAG	ATGGGCTGGA	AAGTCCGTAT	ACTGGGACAA	GTATGATTTG	AGTTGTTTGG	780
GACAAGGACA	GGGGTACAAG	AGAAGGAAAT	GGGCAAAGAG	AGAAGCCTGT	ACTCAGCCAA	840
GGGTGCAGAG	ATGTTATATA	TGATTGCTCT	TCAGGGAACC	GGGCCTCCAG	CTCACACCCC	900
AGCTGCTCAA	CCACCTCCTC	TCTGAATTGA	CTGTCCCTTC	TTTGGAACCTC	TAGGCTTGAC	960
CCCACCTCCCT	GGCCCTCCCA	GCCCACGATT	CCCCTGACCC	GACTCCCTTT	CCCAGAACTC	1020
AGTCGCCTGA	ATCCCCAGCC	TGTGGTTCTC	TCCTAGGCCT	CAGCCTTTCC	TGCCTTTGAC	1080
TGAAACAGCA	GTATCTTCTA	AGCCCTGGGG	GCTTCCCGGG	GCCCCAGCCC	CGACCTAGAA	1140
CCCGCCCGCT	GCCTGCCACG	CTGCCACTGC	CGCTTCTCT	ATAAAGGGAC	CTGAGCGTCC	1200
GGGCCCAGGG	GCTCCGCACA	GCAGGTGAGG	CTCTCCTGCC	CCATCTCCTT	GGGCTGCCCG	1260
TGCTTCGTGC	TTTGGACTAC	CGCCACGACG	TGTCCTGCCC	TCTGCCCTGG	CCTCGGTCCC	1320
TCCTGCACCT	GCTGCCTGGA	TCCCCGGCCT	GCCTGGGCCT	GGGCTTGGTG	GGTTTGGTTT	1380
TGGTTTCCTT	CTCTGTCTCT	GACTCTCCAT	CTGTCACTCT	CATTGTCTCT	GTACACACTT	1440
CTCTGTTTCT	GTATGATTTC	CTCTCTGTTT	CCTTCTGTCT	TCTCTCTGTC	TCCCTCTGCT	1500
CACCTTGGGG	TTTCTCTGAC	TGCATCTTGT	CCCCTTCTCT	GTCGATCTCT	CTCTCGGGGG	1560
TCCGGGGGGT	CTCTCTCCCA	GGGCGGGAGG	TCTGTCTTCC	GCCGCGTGCC	CCGCCCCGCT	1620
CACGTCTCTC	GTATCTCTCT	CTCTTCTCT	GACGGTTCTC	CCCATGACAC	CACCTGAACG	1680
TCTCTTCTCT	CCAAGGGTGT	GTGGCACCAC	CCTACACCTC	CTCCTTCTGG	GGCTGCTGCT	1740
GGTTCGTCTG	CTTGGGGCCC	AGGTGAGGCA	GCAGGAGAAT	GGGGGCTGCT	GGGGTGGCTC	1800
AGCCAAACCT	TGAGCCCTAG	AGCCCCCTC	AACTCTGTTT	TCCCCTAGGG	GCTCCCTGGT	1860
GTTGCCTCA	CACCTTCAGC	TGCCCAGACT	GCCCGTCAGC	ACCCCAAGAT	GCATCTTGCC	1920
CACAGCACCC	TCAAACCTGC	TGCTCACCTC	ATTGGTAAAC	ATCCACCTGA	CCTCCAGAC	1980
ATGTCCCCAC	CAGCTCT					1997

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10240 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GAATCCCCG	GATCAAAGTC	AGCATTAAAT	CCCAGTTTAG	GTTTTGAGGC	TAAGTTCAAG	60
TTTGAAGTCTA	ATGTCAATTT	AGCCTTGTTT	GGAGGACTCA	GAGATTTCAC	TAGTTTCTCC	120
GCAGAGACCA	CTAGAGAAAC	TGCATTTCCC	TGAGTTTGG	GCACAAGACT	CCAGTCATCA	180
CCCCCTCCCAC	ACAGGGAAG	CCCCAAACCA	ACTGCTGGCC	TCCCTAAGAA	AGAAACCGAA	240
TTTACACAA	CCTCCGAAAC	TAAGATTGAA	ACCAAGATTG	GCCCCATCTA	AGGCGCGTCC	300
TCCAGCACAT	TGAGAATGTC	GCTGATGGAG	CCTCGGCCCCA	GCTCTCGAGC	TTCTTCTCTT	360
TCTGTCTCTC	ATGTCCTTCT	ATCACTCCTT	CTCACCTTCC	CGTTTTTGTC	CTGCAATGCC	420
CCCTTCTTCC	TCTCTTCTCT	GGGTTTTTCC	CTTTATTTCT	CACTGTACCA	TTTTATATTT	480
TAATAAAGCC	GAGGTCTCCT	AGTCCATCAG	CTCCTACTGT	TGGAGAGGAG	GCAGAAAGAA	540
ACAGCAGGAC	GGCAAAGGGA	CTCCAGAGAA	AGAGACTCAG	AGGAAAGGCA	AGAAACAGGG	600
ACCAAGAGAG	AGGCCAACAG	TGACACAAGA	CACAGTGAGG	TTAAAAGAAA	TAAGATGAGG	660
CCAAGATAGA	GACCAAGCTA	TTTAAAAGAG	CCATCTGTGG	CTACCCTTCT	TCCGCCATCG	720
CATCTGGTCA	GCCACCAAGA	TTTTGCCTAG	AAACGTTTCT	CCTCTCCATT	CTCCTGCTGC	780
TGCTGCTGCT	GCTGCTGCTG	CTGCTGCTGC	TGCTGCTGCT	GCTGCTGCTG	CTGCCTTAAT	840
ACGAATGCAG	GCTCTTGTCA	TCTCCTTGCT	GGGTTGTTGC	AAAATCCTCC	TAAGTGGTCT	900
CCCACTTCTC	CATTTCCCTC	CCAGCCCCCC	ATCTTCCATA	CTTCCATTTA	TTTATTTTGG	960
CCATGCCCCAT	GGCATGTGGC	AGTTCCAGGG	GCCAGGGATC	AAACCTGTGC	CAATGCAGTG	1020
ACCGTGTGAG	ATCCTTAACC	CACCTGCACAC	AAGGCAACGC	CCCTCGAGTC	ATTCTCATTT	1080
TTTAAATATA	CCAATTGAG	GGGGTCCCTC	TTTCACTTAA	AAATTTTGGC	AGCTCCCTAT	1140
CATGATGAGA	AGGAATTCCA	AACCATTTTT	CTTGTGTGCA	AACCCCTTCA	CATGTGCTCT	1200
CAGCTTACTT	CCCAAGCCTC	ATCCCCTGCTC	CTTCTACGTG	TACCCATGTG	TACATCTCCA	1260
CACACCATAT	ACTCTTTTTT	ACCTCCCATC	TTTGCACCTT	CTGTTCCCTC	TCTCTGCCCC	1320
TCACCATCTT	TTTTGCTTTG	ATACTTAATG	CCTCTCCCTC	AGGCCAGGTT	CAATGGCTTT	1380
TCTGTGGGCT	GCTTTAAGCC	CACCTGTGATG	GAACCTATCA	CATTTTATTT	TATTTGACTT	1440
TCCTTTTAGG	GCCGACCCA	GCAATGGAG	ATTCCCAGGC	TAGGGATCTA	ATCGGAGCTG	1500
TATCTGCCAG	CCTGCGCTGG	AGCCACAGCA	ACGTGGGATC	CGAGCCTGAG	GGGTTTTGAT	1560
GTCTGTGGC	ACAGAAAGTTA	CATTACAGGCT	GTGCATGAAC	TATTTCTCCT	GTTCTCCTCC	1620
CCCTGCTTGA	GGCCCTGCAG	CTTTCCTCT	CATGCCTTGC	TGCTCTGACC	TATGACTTCT	1680

TTTTGTTTGC	ATTCCATCTC	TTTAGTTTTC	TCTCTGTTCC	ACAAACATTT	ACTGAGCATC	1740
TACATGAGGC	ATTGAGGATA	CGGATGGGAA	AGACAGTCCC	CTGACCTCTG	GGACCTCAAA	1800
GACCAATTGT	GGAAGACTGG	TTGGTTATCA	GATAATTACA	ATGAAGTGTG	GGAGTCCCTG	1860
TCATGGGTCA	GCAGGTAATG	AACCCAGTAA	ACGATCCATG	AGGATGCAGA	TTCAATCCCT	1920
GGCCTTGCTC	AGCGGGTTAA	GGATCCAGCG	TTCCCACAAG	CTGTGGTGTA	GGTCGCAGAT	1980
GCGACTCAGA	TCTTGCAATTG	CTGTGGCTGT	GGTGTAGGCT	GGTGGCTACC	CCTAGCCTGG	2040
GAACCTCCAT	ATGCCTCAGG	TGCGGCCCTA	AAAGACAAAA	AAAAAAAAGA	GAGAAACTTT	2100
TCTTTTCTT	AATGTGTAAC	CTACAAGCTA	AGTGAAAACT	GGCTCCTATT	CCATAACGTT	2160
TGTATCATTT	TTCATACTAG	CCAAATACTA	GAAACAGGGA	GTTCCCGTCG	TGGTGCAGCA	2220
GAAACAAATT	CGACTAGGAA	CCATGAGGTT	GCGGGTTGGA	TCCCTGGCCT	TGCTCAGTGG	2280
GTTAAGGATC	CGGCGTTGCC	GTGAGCTGTG	GTGTAGGTCG	CAGATGTGGC	TCGGATCTAG	2340
TGTTGTCTGT	GCCTCTGGTG	AGGCCGCGAG	CAACAGCTCT	GATTAGACTC	CTAGCCTGAG	2400
AACCTCCATA	AGCTGTGGCT	ACGCGCCTAT	AAAGACAAAA	AAAAAAAATA	GGCCAAATAC	2460
TAGAAACAAA	CCAAATGCCC	ATCAACAGAA	GAATAGATAA	GTTAATTGGG	GTATATGCAC	2520
ACAATAGCAT	CACACAATAA	CATGCACACA	ATAACATCAC	AATGAAATAA	AAATTACTAC	2580
TGACAGACAC	AACCATATAG	ATGAATTTCA	CAACACCAAC	AGCGAGAATA	AAAGCCAAGC	2640
ACAGATGAGT	TGTCTGTGTG	GATTCAATTC	TATGAAGTTC	AAGCGCAGGA	AGAACTTAAT	2700
CTATAGTGAC	AGAGGTGAGA	GAGCAGTTGG	TTGTCTTTGG	CAGGTATGAA	CTGGGAGTGG	2760
GCATGAGAGA	ACTTTCTGGA	GACCTAAAAA	TATATTGGAC	TGGATGGTGG	CAACATGGCT	2820
ACAAGAAGAT	GGAAAAGTTC	CTCAGGCTGT	CCACTTGGGA	GACGGGCTTC	TCACGGGACC	2880
TAAGTTCTGC	ATCAGCAGAG	GGGGAAATCC	TTAATGATTT	GACAATTACA	AAGTGTATTG	2940
GCTTTTACCBA	TGTATTTTCA	ACACAATCCC	TCTGCTGTCC	CCACCCACC	CTAGGTCACC	3000
ACCCCTAAGC	TCCACCTGTG	TGGAATTCTG	AAGCCTCCCC	TGTAGAGAAC	TTTAGCAGTT	3060
GCCACGTTCT	TTTGATGTCAG	GAACGTGTTG	TCTAGAGTTA	GACACATCTG	ATCTGTGGGG	3120
CCCACCAAG	TTTGGGACAT	GGTGGGGGCT	GGCCTTCTGC	AGTGAGATGA	AACCTCATTG	3180
TAGGTGATTT	CGTGGCCTCA	TCCCTGAGTC	AGATCTTCCA	AATGAGGACA	CTTTGGAGAG	3240
CAAAAGGGGG	CTCCCTGAAG	ATTTCTCTCA	GGACAGCAGG	AACAAACCAG	GATGTCCCAG	3300
GCAGGAGGGT	ATAGAAGGGA	ACTTGTGTAT	ATGAAATCAG	CCAGATGACC	TGGAATAATC	3360
ACAGACTGGG	ACAAGTGTGA	CTTGAGCCTC	TTGGGCCAG	GACAGGGGTA	CAGAGGAGGA	3420
AACGTGCACA	GAGAGAAGCC	CGTAATCAGC	CAAGGCTGCA	GAGGTGTTAT	ACATAATCGC	3480
TCTTCACGCA	ACCGGGCAAG	CAGCCCACGC	CCCAGCTGCA	CTCCATCTCC	TCCTCTGAAC	3540
TCACCGTCCC	TTCTCTGGAA	CTCCTAAGCC	TGACCCGCT	CCCTGGCCCT	CCCAGCCAC	3600
GGTTCCCGTG	ACCCCACTCC	CTTTCCCAAG	ACTCAGTCAT	CTGAGCCCCC	AGCCTGCGTT	3660
CTCTCCTAGG	CCTCAGCCTT	TCCTGCCTTC	GCGTGAACA	GCAGCATCTT	CTAAGCCCTG	3720
GGCTTCCCCA	GGCCCTAGAC	ACCGCCCTAG	ACCGCCCTAG	CCGACCTGCC	CACGCTGCCA	3780
CTGCCCGCTT	CCTCTATAAA	GGGACCCAGG	GCGCCAGAAA	AGGGGCCAC	AGGGGTCCCC	3840
CACAGCAGGT	GAGACTCTCC	CACCCCATCT	CCTAGGCTG	TCCGGGTGCT	GGACTCCCCC	3900
CTCAGTTCGG	TCCTCCGCTC	CGCTCCCTGG	CCTCTCTGCC	CCTCTGTCAT	CTTCAACCCG	3960
GCCTGGGCTT	TGGTGGGTTT	GGTTTGTGTT	TGTTCTCTCT	GATTCTTTAT	CTGTCAAGGT	4020
CTTTCTAGCT	CTCACACACT	CTGATCCCTC	TCTGTTCCCT	TCCCATCTCT	GTTCCTCTCT	4080
GGGTCTCCCC	CTGCTCACCT	CGGGATTTC	CTGAGTGCCT	CTGGTCCCTT	TCTCTGTCTG	4140
GCGCCCGCTC	TCTGTCTCT	CGGGGTGGCT	GTCTCCGAGG	GCAGGAGGCC	TTCTTCCGCA	4200
GGTGCCCGCG	CCCGCTCACT	GTCTCTCTCC	CCCCACAGGT	TTTCCCCATG	ACACCACCTG	4260
GACGCTCTA	CCTCCGGAGG	GTGTGCAGCA	CCCCCATCCT	CCTCCTCCTG	GGGCTGCTGC	4320
TGGCCCTGCG	GCCCGAGGCC	CAGGTGAGGC	AGCAGGAGAG	CGGGCCGTGG	GGGCAGCCTT	4380
CGCCAACTTT	GGGCCTCAGA	GCCTCTCTGA	CGCTCTCTC	CCCTAGGGGC	TCCCTGGCGT	4440
CGGCCCTCCA	CCCTCAGCTG	CACAGCCTGC	CCATCAGCAC	CCCCCAAAGC	ACTTGGCCAG	4500
AGGCACCTTC	AAACCTGCCG	CTCACCTCGT	TGGTAAACAT	CCACCTGGCC	TCCAGACCT	4560
GTAGCCCCCA	GTCCTCCTCC	TATGCCCTG	CTTCAGGGAC	TGAAGCATCC	CTCCCCCCCC	4620
TCTCCCCCCA	CGCTCAAAAT	GGAGGCATCC	CACCTCCGAC	TCCCTCCCAA	CCATCCCCCA	4680
GGAACTCAGT	CCAGCACCTG	CTTCTCAGG	GATTGAGACC	TCCGACCCCC	AGGTCCTTGA	4740
CTCCCCACCC	CTCTGGCTCT	TCCTAGGAGA	CCCCAGCACC	CCGGACTCAC	TGGCTGGGAG	4800
AGCGAACACG	GATCGTGCCT	TCCTCCGCCA	TGGCTTCTTG	CTGAGCAACA	ACTCCCTGCT	4860
GGTCCCCACC	AGTGGCCTCT	ACTTTGTCTA	CTCCCAGGTC	GTCTTCTCCG	GGGAAGGCTG	4920
CTTCCCCAAG	GCCACCCCCA	CCCTCTCTTA	CCTGGCCAC	GAGGTCCAGC	TCTTCTCCTC	4980
CCAGTACCCC	TTCCACGTGC	CGCTCCTCAG	CGCTCAGAAG	TCCGTGTGCC	CCGGGCCACA	5040
GGGACCTTGG	GTGCGCTCTG	TGTACCAGGG	GGCTGTGTTT	CTGCTCACCC	AGGGAGATCA	5100
GCTGTCCACA	CACACAGAGG	GCACCCCCCA	CCTGTCTCTC	AGCCCCAGTA	GCGTCTTCTT	5160
TGGAGCCTTC	GCTCTATAGA	AGAATCCAGA	AAGAAAAAAA	TGGTTTCAA	GGCCTTCTCC	5220
CCTTTTCAAC	TCCCTTATGA	CCACTTCGGA	GGTCAACGCG	CCTCTCCTCT	GACAAATTTCC	5280
AACAGTCTCA	CTTTCCCCCA	CGCTCAGCAC	CTGGAGCTTC	TGTAGAAGGA	ATTCTAGGCA	5340
CCTCGGGGGA	ACTTGAACCA	CCCCGGATGC	TCTGCTGAGG	ATCTGAATGC	CCGCTTGGAG	5400
CCCTTCCCTT	GTCCTGCCCG	TCTAGGGGCC	CTCGTCCAGG	ACGTGGAAGG	GAAGCTGACC	5460
CATGAGGGAG	TTTGAACGGA	TGACCGGAGC	GGTGTGGGGG	GGTTATTAT	GAAGGGGAAA	5520
ATTAAATTAT	TATTTATGG	AGGATGGAGA	GAAGGGAATC	ACAGAGGAT	GTGAGAAGAG	5580
TGTGACACAT	GTGCCCAAGA	GATAAAGTGA	CAGAAGGCAT	GGGCTCCAGA	TGACCCGGCC	5640
AGAGAGGGCA	AAGTGGCTCA	GGAAAGGGCT	GCTTGAAGTG	AGGCTCATGA	GGAGACGGCT	5700
GACCTTCGAT	GAAACCCAAT	AAAGCTCTTT	TCTCTGAAAT	GCTGTCTGCT	CGTATCTGTC	5760
ACTCGGAGG	GAAAGATTCT	CCAGATGTCT	CTAAGGAGTG	GAGGGAGGAC	AGGAATCAGA	5820
GGGGACGGGA	GCTGTGGGTG	TGTGATGAGG	CCTAAGGGGC	TCAGGTGAGA	GATGGCGGCC	5880
TGAGGGTGAG	GGCAGCCAGA	CCCCTGCAGG	AGAAGCAGAT	GGTTCCTCTG	AGAAGACAAA	5940
GGAAGAGATG	CAGGGCCAAG	GTCTTGAGAA	CCGAGGTCCG	GGGTCCGCTG	GCAGATATGG	6000
CCACAGGTAG	AGGGACAGAG	GAATAGGGGT	GACAGGAGGC	TTCCCGGGAG	AAGGGAAACAC	6060
ACTGAGGGGT	GTTCCGGGAT	CTGAGGGAGG	AGCACGGGGA	CGCCCTGGGA	GACATGCCGT	6120

CCAGGGCCAT	GAGGAGTGGG	AGAGCCTCTG	AGGCTAGCGG	CTGGAGATAC	AGGGACATTT	6180
GAGGAGACAC	GGTCATGGCC	AGGAGCCGCG	AGGGCCTGGA	CAGTCTCTAG	GAATCTCGAA	6240
GAAGCAGGAA	TTCTTTGAGG	ATACGTGGCC	ACACAAAGGG	AGGCTGAGGT	GTGGGGACTT	6300
CATGCAGAAG	TCAGGGCCTC	ACATTTCCCTT	GGAGCCGAG	ACTGAAACCA	GCAGCAGAGT	6360
TTTGGTGAAT	TCCTGTGAGA	GTGAAAGGAG	AAGGCCCGCC	ATGGTGGGTT	TGTGAATTCC	6420
CAGCCTGGCT	TCCTCTCCCT	CTGGGGCTGT	CCAGGCCTG	TTCTTGCCGT	CCTCCCCCAG	6480
CCCGTGTAGG	GCCTCCAGCT	GCCCTTCTCT	CAGCTCCTCT	TCCCTCCAGG	AGACGAAACA	6540
TGGGTCTCAG	CACCCAGCGC	GGTGTCTGTC	AAGTTTTCTC	TCCATTAAGA	ACTCAGCTTT	6600
CTGAAGCTCC	TCCCATTCCT	AGTTCTACCC	CTACCTGAGC	CCTGTTCCGA	AATCAGAGAG	6660
AAATAGAAGT	CATCCCCCAA	AGAAAAGGAA	TTTGTCCCCC	AAAGAAAACAG	AACTTGTCCC	6720
CCAAAGAAAT	GGAAACAATG	GGAAATGGGA	GGCAGGGGGG	ACCTGGGGTC	CAGCCTCCAG	6780
GGTCCTACAC	ACAGAGCAGT	AACTGGCCCA	GCAGCCAC	CTCAGGATCC	GGGCAGGGAG	6840
GGTAGGAAGT	ATCCCTGATG	CCTGGGTGTC	CCCAACTTTC	CAAACCGCCG	CCCCCGCTAT	6900
GGAGATGAAA	CTAAGACAGA	AGGTGACAGG	CCGCTACCG	CTTCTCCAG	ATGAGCTCAT	6960
GGGTTTCTCC	ACCAAGGAAG	TTTTCCGCTG	GTGAAAGAG	AGCCTCTCCC	CGCCCTCTTC	7020
TCACCCAGAG	CGTATAAATG	CAGCTGTTTG	CACACCCAGC	CAGCAGAAGC	TCCCAGAGTG	7080
AGGACACCAG	GGGACACGCC	AGGAGAGAGA	CAAGCCATCT	CCAGGACCCC	CTAGAAATAA	7140
CCTCTCAGAA	GACACACCCC	CGAACAGGCA	GCCGGACGAC	TCTCTCCCTC	TCACACGCTG	7200
CCCCGGGGCG	CCACCATCTC	CCAGCTGGAG	CTGAGCCCCC	CTGAAAAAGA	CACCATGAGC	7260
ACTGAGAGCA	TGATCCGAGA	CGTGGAGCTG	GCGGAGGAGG	CGCTCGCCAA	GAAGGCCGGG	7320
GGCCCCCAGG	GCTCCAGGAG	GTGCCTGTGC	CTCAGCCTCT	TCTCTTCTCT	CCTGGTCCGA	7380
GGAGCCACCA	CGCTCTTCTG	CCTACTGCAC	TTGAGGTTA	TCCGCCCCCA	GAAGGAAGAG	7440
GTGAGCGCCT	GGCCAGCCTT	GGCTCATCT	CCACCCCGGA	GAGAAATGGG	GAAGAAAGAG	7500
GGCCAGAGAC	GAGCTGGGGG	AAAGAAGTGT	GCTGATGGGG	AGTGTGGGGA	GGAAATCATG	7560
GAGAAAGATG	GGGAGGCAGA	AGGAGACGTG	GAGAGAGATG	GGGGGAGAGA	GAGAAGGATG	7620
GAGAGAAATC	CGCTGGCCCG	GCCCTTGGAA	ATGCTCTCTA	AATATTTGTT	GCACGAATGA	7680
GTGAGTAAGC	AGGGACACCC	ATATAAGAG	AGATGAGTAG	ACAGACAAGG	GGTGTGGTAG	7740
AAAGATAGGG	AAAAAACAAG	TGATCTGGAT	AAAGATAGTG	AGACAGGAAG	AGGTAGAGGA	7800
GATAGGAAAG	AGAGATAAGG	AGAGAAGAGG	GAAGCGTGGG	TGTCTGGCAC	GTGGAAGGCA	7860
CTCAATGAAG	GAGTTGTTGA	ATGGATGGGT	GGATGAGAAA	ATGGATGAGT	GGAGAGAAAA	7920
AACTAGACAT	CAGGGCAGAG	AGTACAGCT	AGAGAAGCAG	GTGGCTGTTT	TCCCTTCAGA	7980
GGGGACTTAT	TCAAATCTAA	TTAATCCTTC	TTCTTCTCCC	CAACAGTTTC	CAGCTGGCCC	8040
CTTGAGCATC	AACCCCTCTG	CCCAAGGACT	CAGTAAGTAT	CTCTAAAACC	TGTCTCTCAG	8100
TTCTGAGCTT	GGACAGGGGT	GGGGTTAGTG	CTGGGGTGGG	AGGAAGAAGG	GAAATTTAGG	8160
GTTCTGGGTTT	AGTCGGGGGA	ATGCAGTCA	AAGTAGTGAG	ATATTTTCTG	GGAAGTCTGA	8220
GGGTCTCATC	TTTTTCTTTT	CTCTTTCTCT	CTCAGGATCA	TCGTCTCAAA	CCTCAGATAA	8280
GCCCGTCCGC	CACGTTGTAG	GTAAGAGTTC	TGAGGATGTG	TCTGGGGGAT	GAAGAAATAG	8340
GCAGGACAGA	GAGGGTAGG	ATTTGGGGGC	TGAAGCCAGG	CTGAGGGTAG	CCAGAGCTTG	8400
GAGATAGTAT	GAGGAGGACT	CGCTGAGCTC	CAGGGGAGGA	TGGGGGATAC	TCAGAACTTG	8460
AGGAGGATAC	TCCGAACCTC	ATGGACAGAT	GGGATGTGGG	AAGACAGACC	GAGGGGACAG	8520
GAACCCGATG	TGGGGGGCGG	GCAGAACTCG	AGGGCCAGGA	TGTGGAGAGT	GGAAGTGAAC	8580
GGGTCACTACT	GACTCACCCC	TCCCTCTTTG	TCTCTCCCT	CCAGCCAATG	TCAAAGCCGA	8640
GGGACAGCTC	CAATGGCAGA	GTGGGTATGC	CAATGCCCTC	CTGGCCAACG	GCGTGAAGCT	8700
GAAAGACAAC	CAGCTGGTGG	TGCCGACAGA	TGGGCTGTAC	CTCATCTACT	CCCAGGTCCT	8760
CTTCAGGGGC	CAAGGCTGCC	CTTCCACCAA	CGTTTTCTCT	ACTCACACCA	TCAGCCGCAT	8820
CGCCGTCTCC	TACCAGACCA	AGGTCAACCT	CCTCTCTGCC	ATCAAGAGCC	CTTGCCAGAG	8880
GGAGACCCCC	GAGGGGGCCG	AGGCCAAGCC	CTGGTACGAA	CCCATCTACC	TGGGAGGGGT	8940
CTTCCAGCTG	GAGAAGGATG	ATCGACTCAG	TGCCGAGATC	AACCTGCCCG	ACTATCTGGA	9000
CTTTGCTGAA	TCTGGGCAGG	TCTATTTTGG	GATCATTGCC	CTGTGAGGGG	GCAGGACATC	9060
CGTTCCCTCC	CCTGTCCATC	CCTTTATTAT	TTTACTCCTT	CAGACCCCTT	CACGTCCTTC	9120
TGGTTTAGAA	AGAGAATGAG	GGGCTGGGGA	CTGGGCTCCA	AGCTTAAAC	TTTAAACAAC	9180
AACAGCAACA	CTTAGAAATC	AGGGATTGAG	GGATGTGTGG	CCTGGACAAC	CAGGCACTGA	9240
CCACCACCAA	GAATTGGAAC	TGGGGCTTCC	AGACTCGCTG	GGGTCTTGG	GTTTGGATTG	9300
CTGGATGCAA	CCTGGGACAT	CTGGAATGTG	GCTGCCAGGG	AAGCTTGGGT	TCCAATCGGA	9360
ATACTTCAGA	ACATTCCTTG	AGAAGATTTC	ACCTCAATCT	TGATGACTTT	TTAGGCTTCC	9420
CTTTCTTCCA	ATTTTCCAGA	CTTCCCTGGG	ATGGGGAGCC	CAGCCCCAAA	CCCCACAGGC	9480
CAGCTCCCTC	TTATTTATAT	TTGCACTTGG	CATTATTATT	TATTTATTTA	TTTATTATTT	9540
ATTTACTAGT	GAATGTATTT	ATTGAGGAGG	GCGAGGTGTC	CTGGGAGACC	CAGCATAAGG	9600
GCTGCCTTGG	TTGAGATGTG	TTTTCTGTGA	AAACGGAGCT	GAACTGTAGG	TTGCTCCAC	9660
CTGGCCTCCT	AGCCTCTGTG	CCTCCTTTTG	CTTATGTTTT	TAAAAACAAA	TATTTATCTG	9720
ATCGAGTTGT	CTAAATAATG	CTGATTGGT	GACTAACTTG	TCGTACATC	GCTGAACCTC	9780
TGCTCCCCAG	GGGAGTTGTG	TCTGTAACCG	CCCTACTGGT	CAGTGGCGAG	AAATAAAGC	9840
GTGCTTAGAA	AGAAATCTG	GCGACTGAAT	TCTGCATCTC	TCTGCATCTC	CTTGGGGGGG	9900
TGAGGCTGCT	CCCCAAAATT	CTTTCTCCAC	CGGGCTTAGG	ATTCCCTGGG	CTTCACTCCT	9960
GAGCTTGGAC	TGCTTGGCTC	AGGAGCCTCT	GCAAGAAACA	AAGCCAGCC	AAACAGGTCC	10020
CTCCCCTAAG	AAAGGAACCT	ACCTCTCCCT	CAGGGTGTGG	GAGGGTGTGG	GAATTTCCAA	10080
GTCTGGGAAT	TCCTATCCAG	CTGGGGAAGT	CTGCAGTGCA	GGTGAGACTT	CCGGCTGAAA	10140
GAGCCAGGGA	GCGGCCAGAT	GCTCAGGTAC	CTGAACCAGA	GCCAGGGGAC	TTCCAGACAG	10200
TGAGGCAACT	GGGCTCCAAA	TAACCTGATC	CGGGGAATTC			10240

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(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1644 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCTCAGCGAG	GACAGCAAGG	GACTAGCCAG	GAGGGAGAAC	AGAAACTCCA	GAACATCCTG	60
GAAATAGCTC	CCAGAAAAGC	AAGCAGCCAA	CCAGGCAGGT	TCTGTCCCTT	TCACTCACTG	120
GCCCAAGGCG	CCACATCTCC	CTCCAGAAAA	GACACCATGA	GCACAGAAAG	CATGATCCGC	180
GACGTGGAAC	TGGCAGAAGA	GGCACTCCCC	CAAAAGATGG	GGGGCTTCCA	GAACCTCCAG	240
CGGTGCCTAT	GTCTCAGCCT	CTTCTCATTC	CTGCTTGTGG	CAGGGGCCAC	CACGCTCTTC	300
TGTCTACTGA	ACTTCGGGGT	GATCGGTCCC	CAAAGGGATG	AGAAGTTCCC	AAATGGCCTC	360
CCTCTCATCA	GTTCTATGGC	CCAGACCCCTC	ACATCAGAT	CATCTTCTCA	AAATTGAGT	420
GACAAGCCTG	TAGCCACAGT	CGTAGCAAAC	CACCAAGTGG	AGGAGCAGCT	GGAGTGGCTG	480
AGCCAGCGCG	CCAACGCCCT	CCTGGCCAAC	GGCATGGATC	TCAAAGACAA	CCAAC TAGTG	540
GTGCCAGCCG	ATGGGTGTGA	CCTTGTCTAC	TCCCAGGTTT	TCTTCAAGGG	ACAAGGCTGC	600
CCCGACTACG	TGCTCCTCAC	CCACACCGTC	AGCCGATTG	CTATCTCATA	CCAGGAGAAA	660
GTCAACCTCC	TCTCTGCCGT	CAAGAGCCCC	TGCCCAAGG	ACACCCCTGA	GGGGGCTGAG	720
CTCAAACCCT	GGTATGAGCC	CATATACTCG	GGAGGAGTCT	TCCAGCTGGA	GAAGGGGGAC	780
CAACTCAGCG	CTGAGGTCAA	TCTGCCCAAG	TACTTAGACT	TTGCGGAGTC	CGGGCAGGTC	840
TACTTTGGAG	TCATTGCTCT	GTGAAGGGAA	TGGGTGTTCA	TCCATTCTCT	ACCCAGCCCC	900
CACTCTGACC	CCTTTACTCT	GACCCCTTTA	TTGTCTACTC	CTCAGAGCCC	CCAGTCTGTG	960
TCCTTCTAAC	TTAGAAAGGG	GATTATGGCT	CAGAGTCCAA	CTCTGTGCTC	AGAGCTTTCA	1020
ACAATACTC	AGAAACACAA	GATGCTGGGA	CAGTGACCTG	GACTGTGGGC	CTCTCATGCA	1080
CCACCATCAA	GGACTCAAAT	GGGCTTTCCG	AATCACTGG	AGCCTCGAAT	GTCATTCTCT	1140
GAGTTCTGCA	AAGGGAGAGT	GGTCAGGTTG	CCTCTGTCTC	AGAATGAGGC	TGGATAAGAT	1200
CTCAGGCCCT	CTTACCTTCA	GACCTTTCCA	GACTCTTCCC	TGAGGTGCAA	TGCACAGCCT	1260
TCCTCACAGA	GCCAGCCCCC	CTCTATTAT	ATTGCACTT	ATTATTTATT	ATTTATTTAT	1320
TATTTATTTA	TTTGCTTATG	AATGTATTTA	TTTGAAGGC	CGGGGTGTCC	TGGAGGACCC	1380
AGTGTGGGAA	GCTGTCTTCA	GACAGACATG	TTTCTGTGA	AAACGGAGCT	GAGCTGTCCC	1440
CACCTGGCCT	CTCTACCTTG	TTGCCTCCTC	TTTTGCTTAT	GTTTAAACAA	AAATATTTAT	1500
CTAACCCAAT	TGTCTTAATA	ACGCTGATTT	GGTGACCAGG	CTGTCGCTAC	ATCACTGAAC	1560
CTCTGCTCCC	CACGGGAGCC	GTGACTGTAA	TTGCCCTACA	GTCAATTGAG	AGAAATAAAG	1620
ATCGCTTAAA	ATAAAAAACC	CCCC				1644

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1890 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AAACAGAGAG	AGATAGAGAA	AGAGAAAGAC	AGAGGTGTTT	CCCTTAGCTA	TGGAAACTCT	60
ATAAGAGAGA	TCCAGCTTGC	CTCCTCTTGA	GCAGTCAGCA	ACAGGGTCCC	GTCCTTGACA	120
CCTCAGCCTC	TACAGGACTG	AGAAGAAGTA	AAACCGTTTG	CTGGGGCTGG	CCTGACTCAC	180
CAGCTGCCAT	GCAGCAGCCC	TTCAATTACC	CATATCCCCA	GATCTACTGG	GTGGACAGCA	240
GTGCCAGCTC	TCCCTGGGCC	CCTCCAGGCA	CAGTTCTTCC	CTGTCCAACC	TCTGTGCCCA	300
GAAGGCGCTG	TCAAAGGAGG	CCACCACCAC	CACCGCCACC	GCCACCACTA	CCACCTCCGC	360
CGCCGCCGCC	ACCCTGCCCT	CCACTACCGC	TGCCACCCCT	GAAGAAGAGA	GGAACCCACA	420
GCACAGGCCT	GTGTCTCCTT	GTGATGTTT	TCATGGTTCT	GGTTGCCTTG	GTAGGATTGG	480
GCCTGGGGAT	GTTTCAGCTC	TTCCACCTAG	AGAAGGAGCT	GGCAGAACTC	CGAGAGTCTA	540
CCAGCCAGAT	GCACACAGCA	TCATCTTTGG	AGAAGCAAAT	AGGCCACCCC	AGTCCACCCC	600
CTGAAAAAAA	GGAGCTGAGG	AAAGTGGCCC	ATTAAACAGG	CAAGTCCAAC	TCAAGGTCCA	660
TGCCTTGGGA	ATGGGAAGAC	ACCTATGGAA	TTGTCTTGCT	TTCTGGAGTG	AAGTATAAGA	720
AGGGTGGCCT	TGTGATCAAT	GAAACTGGGC	TGTACTTTGT	ATATTCCAAA	GTATACTTCC	780
GGGGTCAATC	TTGCAACAAC	CTGCCCCCTGA	GCCACAAGGT	CTACATGAGG	AACTCTAAGT	840

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ATCCCCAGGA	TCTGGTGATG	ATGGAGGGGA	AGATGATGAG	CTACTGCACT	ACTGGGCAGA	900
TGTGGGCCCC	CAGCAGCTAC	CTGGGGGCG	TGTTCAATCT	TACCAGTGCT	GATCATTAT	960
ATGTCAACGT	ATCTGAGCTC	TCTCTGGTCA	ATTTTGAGGA	ATCTCAGACG	TTTTTCGGCT	1020
TATATAAGCT	CTAAGAGAAG	CACTTTGGGA	TTCTTTCCAT	TATGATTCTT	TGTTACAGGC	1080
ACCGAGAATG	TTGTATTTCAG	TGAGGGTCTT	CTTACATGCA	TTTGAGGTCA	AGTAAGAAGA	1140
CATGAACCAA	GTGACCTTGG	AGACCACAGG	GTTCAAAATG	TCTGTAGCTC	CTCAACTCAC	1200
CTAATGTTTA	TGAGCCAGAC	AAATGGAGGA	ATATGACGGA	AGAACATAGA	ACTCTGGGCT	1260
GCCATGTGAA	GAGGGAGAAG	CATGAAAAAG	CAGCTACCCA	GGTGTCTTAC	ACTCATCTTA	1320
GTGCCTGAGA	GTATTTAGGC	AGATTGAAAA	GGACACCTTT	TAACTCACCT	CTCAAGGTGG	1380
GCCTTGCTAC	CTCAAGGGGG	ACTGTCTTTC	AGATACATGG	TTGTGACCTG	AGGATTTAAG	1440
GGATGGAAAA	GGAGACTAG	AGGCTTGCAT	AATAAGCTAA	AGAGGCTGAA	AGAGGCCAAT	1500
GCCCCACTGG	CAGCATCTTC	ACTTCTAAAT	GCAATATCCTG	AGCCATCGGT	GAAACTAACA	1560
GATAAGCAAG	AGAGATGTTT	TGGGGACTCA	TTTCATTCTT	AACACAGCAT	GTGTATTTC	1620
AGTGCCCAATT	GTAGGGGTGT	GTGTGTGTGT	GTGTGTGTGT	GTGTGTGTGT	AAAGAGAGAA	1680
TGTAGATATT	GTGAAGTACA	TATTAGGAAA	ATATGGGTGT	CATTGGGTCA	AGATTTTGAA	1740
TGCTTCTCGA	CAATCAACTC	TAATAGTGCT	TAAAAATCAT	TGATTGTGAG	CTACTAATGA	1800
TGTTTTCTTA	TAATATAATA	AATATTTATG	TAGATGTGCA	TTTTTGTGAA	ATGAAAACAT	1860
GTAATAAAAA	GTATATGTTA	GGATACAAAT				1890

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1541 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGGTGTCTCA	CAGAGAAGCA	AAGAGAAGAG	AACAGGAGAA	ATGGTGTTC	CCTTGACTGC	60
GGAAACTTTA	TAAAGAAAAC	TTAGCTTCTC	TGGAGCAGTC	AGCGTCAGAG	TTCTGTCCCT	120
GACACCTGAG	TCTCCTCCAC	AAGGCTGTGA	GAAGGAAAAC	CTTTCCTGGG	GCTGGGTGCC	180
ATGCAGCAGC	CCATGAATTA	CCCATGTCCC	CAGATCTTCT	GGGTAGACAG	CAGTGCCACT	240
TCATCTTGGG	CTCCTCCAGG	GTCAGTTTTC	CCCTGTCCAT	CTTGTGGGCC	TAGAGGGCCG	300
GACCAAAGGA	GACCGCCACC	TCCACCACCA	CCTGTGTGTC	CCTACCACC	GCCATCACAA	360
CCACTCCAC	TGCGGCCACT	GACCCCTCTA	AAGAAGAAGG	ACCACAACAC	AAATCTGTGG	420
CTACCGGTGG	TATTTTTCAT	GGTCTCTGTC	GCTCTGGTTG	GAATGGGATT	AGGAATGTAT	480
CAGCTCTTCC	ACCTGCAGAA	GGAAGTGGCA	GAATCCGTCG	AGTTACCAA	CCAAAGCCTT	540
AAAGATATCAT	CTTTTGAAAA	GCAATAGGCC	AACCCCACTA	CACCTCTGTA	AAAAAAGAG	600
CCGAGGAGTG	TGGCCCATTT	AACAGGGAAC	CCCCACTCAA	GGTCCATCCC	TCTGGAATGG	660
GAAGACACAT	ATGGAACCGC	TCTGATCTCT	GGAGTGAAGT	ATAAGAAAGG	TGGCCTTGTC	720
ATCAACGAAA	CTGGGTGTGA	CTTCGTGTAT	TCCAAAGTAT	ACTTCCGGGG	TCAGTCTTGC	780
AACAACCAGC	CCCTAAACCA	CAAGGTCTAT	ATGAGGAACT	CTAAGTATCC	TGAGGATCTG	840
GTGCTAATGG	AGGAGAAGAG	GTTGAACACT	TGCACTACTG	GCCAGATATG	GGCCACAGC	900
AGCTACCTGG	GGGCAGTATT	CAATCTTACC	AGTGTGACCT	ATTTATATGT	CAACATATCT	960
CAACTCTCTC	TGATCAATTT	TGAGGAATCT	AAGACCTTTT	TCGGCTTGTA	TAAGCTTTAA	1020
AAGAAAAGC	ATTTTAAAT	GATCTACTAT	TCTTTATCAT	GGGCACCAGG	AAATATTGCT	1080
TGAATGAGAG	TCTTCTTAAG	ACCTATTGAG	ATTAATTAAG	ACTACATGAG	CCACAAAGAC	1140
CTCATGACCG	CAAGGTCCAA	CAGGTCAGCT	ATCCTTCATT	TTCTCGAGGT	CCATGGAGTG	1200
GTCCTTAATG	CCTGCATCAT	GAGCCAGATG	GAAGGAGGTC	TGTGACTGAG	GGACATAAAG	1260
CTTTGGGCTG	CTGTGTAGCA	ATGCAGAGGC	ACAGAGAAAG	AACTGTCTGA	TGTTAAATGG	1320
CCAAGAGAA	TTTAACCATT	GAAGAAGACA	CCTTTACACT	CACCTCCAGG	GTGGGTCTAC	1380
TTACTACCTC	ACAGAGGCCG	TTTTTGAGAC	ATAGTTGTGG	TATGAATATA	CAAGGGTGAG	1440
AAAGGAGGCT	CATTGACTG	ATAAGCTAGA	GACTGAAAAA	AAGACAGTGT	CTCATTGGCA	1500
CCATCTTTAC	TGTTACCTGA	TGTTTTCTGA	GCCGACCTTT	G		1541

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 888 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GGCTGGTCCC	CTGACAGGTT	GAAGCAAGTA	GACGCCCAGG	AGCCCCGGGA	GGGGGCTGCA	60
GTTTCCTTCC	TTCCTTCTCG	GCAGCGCTCC	GCGCCCCCAT	CGCCCCCTCT	GCGCTAGCGG	120
AGGTGATCGC	CGCGGCGATG	CCGGAGGAGG	GTTGGGGCTG	CTCGGTGCGG	CGCAGGCCCT	180
ATGGGTGCGT	CCTGCGGGCT	GCTTTGGTCC	CATTGGTCGC	GGGCTTGGTG	ATCTGCCTCG	240
TGGTGTGCAT	CCAGCGCTTC	GCACAGGCTC	AGCAGCAGCT	GCCGCTCGAG	TCACTTGGGT	300
GGGACGTAGC	TGAGCTGCAG	CTGAATCACA	CAGGACCTCA	GCAGGACCCC	AGGCTATACT	360
GGCAGGGGGG	CCCAGCACTG	GGCCGCTCCT	TCCTGCATGG	ACCAGAGCTG	GACAAGGGGC	420
AGCTACGTAT	CCATCGTGAT	GGCATCTACA	TGGTACACAT	CCAGGTGACG	CTGGCCATCT	480
GCTCCTCCAC	GACGGCCTCC	AGGCACCACC	CCACCACCCT	GGCCGTGGGA	ATCTGCTCTC	540
CCGCTCCCCG	TAGCATCAGC	CTGCTGCGTC	TCAGCTTCCA	CCAAGTTTGT	ACCATTGCTT	600
CCCAGCGCCT	GACGCCCCCT	CCCCGAGGGG	ACACACTCTG	CACCAACCTC	ACTGGGACAC	660
TTTTCCTTTC	CGGAAACACT	GATGAGACCT	TCTTTGGAGT	GCAGTGGGTG	CGCCCCCTGAC	720
CACGTGCTGT	CATTAGGGTT	TTTTAAATTT	TATTTTATTT	TATTTAAGTT	CAAGAGAAAA	780
AGTGTACACA	CAGGGGCCAC	CCGGGGTTGG	GGTGGGAGTG	TGGTGGGGGG	TAGTGGTGGC	840
AGGACAAGAG	AAGGCATTGA	GCTTTTTCTT	TCATTTTCCT	ATTAAAAA		888

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1906 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CCAAGTCACA	TGATTACAGGA	TTCAGGGGGA	GAATCCTTCT	TGGAACAGAG	ATGGGCCCCAG	60
AACCTGAATCA	GATGAAGAGA	GATAAGGTGT	GATGTGGGGA	AGACTATATA	AAGAATGGAC	120
CCAGGGCTGC	AGCAAGCACT	CAACGGAATG	GCCCCTCCTG	GAGACACAGC	CATGCATGTG	180
CCGGCGGGCT	CCGTGGCCAG	CCACCTGGGG	ACCACGAGCC	GCAGCTATTT	CTATTTGACC	240
ACAGCCACTC	TGGCTCTGTG	CCTTGTCTTC	ACGGTGGCCA	CTATTATGGT	GTGGTTCGTT	300
CAGAGGACGG	ACTCCATTCC	CAACTCACCT	GACAACGTCC	CCCTCAAAGG	AGGAAATGTC	360
TCAGAAGACC	TCTTATGTAT	CCTGAAAAGA	GCTCCATTCA	AGAAGTCATG	GGCCTACCTC	420
CAAGTGGCAA	AGCATCTAAA	CAAAACCAAG	TTGTCTTGGA	ACAAAGATGG	CATTCTCCAT	480
GGAGTCAGAT	ATCAGGATGG	GAATCTGGTG	ATCCAATTCC	CTGGTTTGTA	CTTCATCATT	540
TGCCAACTGC	AGTTTCTTGT	ACAATGCCCA	AATAATTCTG	TCGATCTGAA	GTTGGAGCTT	600
CTCATCAACA	AGCATATCAA	AAAACAGGCC	CTGGTGACAG	TGTGTGAGTC	TGGAATGCAA	660
ACGAAACACG	TATACCAGAA	TCTCTCTCAA	TTCTTGCTGG	ATTACCTGCA	GGTCAACACC	720
ACCATATCAG	TCAATGTGGA	TACATTCCAG	TACATAGATA	CAAGCACCTT	TCCTCTTGAG	780
AATGTGTTGT	CCATCTTCTT	ATACAGTAAT	TCAGACTGAA	CAGTTTCTCT	TGGCCTTCAG	840
GAAGAAAGCG	CCTCTCTACC	ATACAGTATT	TCATCCCTCC	AAACACTTGG	GCAAAAAGAA	900
AACCTTAGAC	CAAGACAAAC	TACACAGGGT	ATTAAATAGT	ATACTTCTCC	TTCTGTCTCT	960
TGGAAGAGATA	CAGCTCCAGG	GTTAAAAAGA	GAGTTTTTAG	TGAAGTATCT	TTCAGATAGC	1020
AGGCAGGGAA	GCAATGTAGT	GTGGTGGGCA	GAGCCCCACA	CAGAATCAGA	AGGGATGAAT	1080
GGATGTCCCA	GCCCCAACAC	TAATTCACCTG	TATGGTCTTG	ATCTATTTCT	TCTGTTTTGA	1140
GAGCCTCCAG	TTAAAATGGG	GCTTCAGTAC	CAGAGCAGCT	AGCAACTCTG	CCCTAATGGG	1200
AAATGAAGGG	GAGCTGGGTG	TGAGTGTTTA	CACTGTGCCC	TTCACGGGAT	ACTTCTTTTA	1260
TCTGCAGATG	GCCTAATGCT	TAGTTGTCCA	AGTCGCGATC	AAGGACTCTC	TCACACAGGA	1320
AACTTCCCTA	TACTGGCAGA	TACACTTGTG	ACTGAACCAT	GCCCAGTTTA	TGCCTGTCTG	1380
ACTGTCACTC	TGGCACTAGG	AGGCTGATCT	TGTACTCCAT	ATGACCCAC	CCCTAGGAAC	1440
CCCCAGGGAA	AACCAAGGCTC	GGACAGCCCC	CTGTTCCCTGA	GATGGAAAGC	ACAAATTAA	1500
TACACCACCA	CAATGGAAAA	CAAGTTCAAA	GACTTTTACT	TACAGATCCT	GGACAGAAAG	1560
GGCATAATGA	GTCTGAAGGG	CAGTCCTCCT	TCTCCAGGTT	ACATGAGGCA	GGAATAAGAA	1620
GTACAGACAGA	GACAGCAAGA	CAGTTAAACA	CGTAGGTAAG	GAAATAGGGT	GTGGTCACTC	1680
TCAATTCATC	GGCAAATGCC	TGAATGGTCT	GTCTGAAGGA	AGCAACAGAG	AAGTGGGGAA	1740
TCCAGTCTGC	TAGGCAGGAA	AGATGCCCTC	AAGTTCTTGT	CTCTGGCCAG	AGGTGTGGTA	1800
TAGAACCAGA	AACCCATATC	AAGGGTGAAT	AAGCCCGGCT	TCCGTATAGA	GAAATTAAAC	1860
TTGTATACAA	AATGGTTGCC	AAGGCAACAT	AAAATTATAA	GAATTC		1906

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(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1619 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GTCATGGAAT	ACGCTCTGA	CGCTTCACTG	GACCCCGAAG	CCCCGTGGCC	TCCCGCGCCC	60
CGCGCTCGCG	CCTGCCGCGT	ACTGCCTTGG	GCCCTGGTCG	CGGGGCTGCT	GCTGCTGCTG	120
CTGCTCGCTG	CCGCTGCGC	CGTCTTCCTC	GCCTGCCCTT	GGGCCGTGTC	CGGGGCTCGC	180
GCCTCGCCCG	GCTCCGCGGC	CAGCCCGAGA	CTCCGCGAGG	GTCCCGAGCT	TTCGCCCGAC	240
GATCCCGCCG	GCCTCTTGGG	CCTGCGGCAG	GGCATGTTTG	CGCAGCTGGT	GGCCCAAAAT	300
GTTCTGCTGA	TGATGGGGCC	CCTGAGCTGG	TACAGTGACC	CAGGCCTGGC	AGGCGTGTCC	360
CTGACGGGGG	GCCTGAGCTA	CAAAGAGGAC	ACGAAGGAGC	TGGTGGTGGC	CAAGGCTGGA	420
GTCTACTATG	TCCTCTTTCA	ACTAGAGCTG	CGGCGCGTGG	TGGCCGGCGA	GGGCTCAGGC	480
TCCGTTTCAC	TTGCGCTGCA	CCTGCAGCCA	CTGCGCTCTG	CTGCTGGGGC	CGCCGCCCTG	540
GCTTTGACCG	TGGACCTGCC	ACCCGCCTCC	TCCGAGGCTC	GGAACCTCGC	CTTCGGTTTC	600
CAGGGCCGCT	TGCTGCACCT	GAGTGCCGGC	CAGCGCCTGG	GCCTCCATCT	TCACACTGAG	660
GCCAGGGCAC	GCCATGCCTG	GCAGCTTACC	CAGGGCGCCA	CAGTCTTGGG	ACTCTTCCGG	720
GTGACCCCCG	AAATCCCGAG	CGGACTCCCT	TCACCGAGGT	CGGAATAACG	CCCAGCCTGG	780
GTGCAGCCCA	CCTGGACAGA	GTCCGAATCC	TACTCCATCC	TTCATGGAGA	CCCCTGGTGC	840
TGGGTCCCTG	CTGCTTTCTC	TACCTCAAGG	GGCTTGGCAG	GGGTCCCTGC	TGCTGACCTC	900
CCCTTGAGGA	CCCTCCTCAC	CCACTCCTTC	CCCAAGTTGG	ACCTTGATAT	TTATTCTGAG	960
CCTGAGCTCA	GATAATATAT	TATATATATT	ATATATATAT	ATATATTTCT	ATTTAAAGAG	1020
GATCCTGAGT	TTGTGAATGG	ACTTTTTTAG	AGGAGTTGTT	TTGGGGGGGG	GGTCTTCGAC	1080
ATTGCCGAGG	CTGTCTTTGA	ACTCCTGGAC	TTAGACGATC	CTCCTGCCTC	AGCCTCCCAA	1140
GCAACTGGGA	TTGATCCTTT	CTATTAAATC	ATTGTACTTA	TTTGCTTATT	TGTGTGTATT	1200
GAGCATCTGT	AATGTGCCAG	CATTGTGCCC	AGGCTAGGGG	GCTATAGAAA	CATCTAGAAA	1260
TAGACTGAAA	GAAATCTGTA	GTTATGGTAA	TACGTGAGGA	ATTTAAAGAC	TCATCCCCAG	1320
CTGCCACCTC	CTGTGTGATA	CTTGGGGGCT	AGCTTTTTTC	TTTCTTTCTT	TTTTTTGAGA	1380
TGGTCTTGTT	CTGTCAACCA	GGCTAGAATG	CAGCGGTGCA	ATCATGAGTC	AATGCAGCCT	1440
CCAGCCTCGA	CCTCCCGAGG	CTCAGGTGAT	CCTCCCATCT	CAGCCTCTCG	AGTAGCTGGG	1500
ACCACAGTTG	TGTGCCACCA	CACTTGGCTA	ACTTTTTAAT	TTTTTTGCGG	AGACGGTATT	1560
GCTATGTTGC	CAAGGTTGTT	TACATGCCAG	TACAATTTAT	AATAAACACT	CATTTTTTCC	1619

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1239 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AGCCTATAAA	GCACGGGCAC	TGGCGGGAGA	CGTGCACTGA	CCGACCGTGG	TAATGGACCA	60
GCACACACTT	GATGTGGAGG	ATACCGCGGA	TGCCAGACAT	CCAGCAGGTA	CTTCGTGCCC	120
CTCGGATGCG	GCGCTCCTCA	GAGATACCGG	GCTCCTCGCG	GACGCTGCGC	TCCTCTCAGA	180
TACTGTGCGC	CCCACAAATG	CCGCGCTCCC	CACGGATGCT	GCCTACCCTG	CGGTTAATGT	240
TCGGGATCGC	GAGGCCGCGT	GGCCGCTGTC	ACTGAACTTC	TGTTCCCGCC	ACCCAAAGCT	300
CTATGGCCTA	GTCGCTTTGG	TTTTGTGCTT	TCTGATCGCC	GCCTGTGTTT	CTATCTTCAC	360
CCGCACCGAG	CCTCGGCCAG	CGCTCACAAT	CACCACCTCG	CCCAACCTGG	GTACCCGAGA	420
GAATAATGCA	GACCAGGTCA	CCCCTGTTTC	CCACATTGGC	TGCCCAACA	CTACACAACA	480
GGGCTCTCCT	GTGTTGCGCA	AGCTACTGGC	TAAAAACCAA	GCATCGTTGT	GCAATACAAC	540
TCTGAAGTGG	CACAGCCAAAG	ATGGAGCTGG	GAGCTCATAC	CTATCTCAAG	GTCTGAGGTA	600
CGAAGAAGAC	AAAAAGGAGT	TGGTGGTAGA	CAGTCCCGGG	CTCTACTACG	TATTTTGGGA	660
ACTGAAGCTC	AGTCCAACAT	TCACAAACAC	AGGCCACAAG	GTGCAGGGCT	GGGTCTCTCT	720
TGTTTTGCAA	GCAAAGCCTC	AGGTAGATGA	CTTTGACAAC	TTGGCCCTGA	CAGTGGAACT	780
GTTCCCTTGC	TCCATGGAGA	ACAAGTTAGT	GGACCGTTCC	TGGAGTCAAC	TGTTGCTCCT	840
GAAGGCTGGC	CACCGCTCA	GTGTGGGTCT	GAGGGCTTAT	CTGCATGGAG	CCCAGGATGC	900

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ATACAGAGAC	TGGGAGCTGT	CTTATCCCAA	CACCACCAGC	TTTGGACTCT	TTCTTGTGAA	960
ACCCGACAAC	CCATGGGAAT	GAGAACTATC	CTTCTTGTGA	CTCCTAGTTG	CTAAGTCCTC	1020
AAGCTGCTAT	GTTTTATGGG	GTCTGAGCAG	GGGTCCCTTC	CATGACTTTC	TCTTGTCTTT	1080
AACTGGACTT	GGTATTTATT	CTGAGCATAG	CTCAGACAAG	ACTTTATATA	ATTCACTAGA	1140
TAGCATTAGT	AAACTGCTGG	GCAGCTGCTA	GATAAAAAAA	AATTTCATAA	TCAAAGTTTA	1200
TATTTATATT	AATATATAAA	AATAAATGTG	TTTGTAAT			1239

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 606 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTTCTTATCA	CCCAGATGAT	TGGGTCAGCA	120
CTTTTGTCTG	TGTATCGCTT	CGCACAGGCT	TTTGAAATGC	AAAAAGGTGA	TCAGAATCCT	180
CAAATTGCGG	CACATGTCAT	AAGTGAGGCC	AGCAGTAAAA	CAACATCTGT	GTTACAGTGG	240
GCTGAAAAAG	GATACTACAC	CATGAGCAAC	AACTTGGTAA	CCCTGGAAAA	TGGGAAACAG	300
CTGACCGTTA	AAAGACAAGG	ACTCTATTAT	ATCTATGCCC	AAGTCACCTT	CTGTTCCAAT	360
CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAGTC	CCCCGGTAGA	420
TTGAGAGAG	TCTTACTCAG	AGCTGCAAA	ATCCACAGTT	CCGCCAAACC	TTGCGGGCAA	480
CAATCCATTC	ACTTGGGAGG	AGTATTTGAA	TTGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	540
GTGACTGATC	CAAGCCAAGT	GAGCCATGGC	ACTGGCTTCA	CGTCCTTTGG	CTTACTCAAA	600
CTCTGA						606

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 783 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTTCTTATCA	CCCAGATGAT	TGGGTCAGCA	120
CTTTTGTCTG	TGTATCTTCA	TAGAAGATTG	GATAAGGTCG	AAGAGGAAGT	AAACCTTCAT	180
GAAGATTTTG	TATTCATAAA	AAAGCTAAAG	AGATGCAACA	AAGGAGAAGG	ATCTTTATCC	240
TTGCTGAACT	GTGAGGAGAT	GAGAAGGCAA	TTTGAAGACC	TTGTCAAGGA	TATAACGTTA	300
AACAAAGAAG	AGAAAAAAGA	AAACAGCTTT	GAAATGCAAA	AAGGTGATCA	GAATCCTCAA	360
ATTGCGGCAC	ATGTCATAAG	TGAGGCCAGC	AGTAAAACAA	CATCTGTGTT	ACAGTGGGCT	420
GAAAAAGGAT	ACTACACCAT	GAGCAACAAC	TTGGTAACCC	TGGAAAATGG	GAAACAGCTG	480
ACCGTTAAAA	GACAAGGACT	CTATTATATC	TATGCCCAAG	TCACCTTCTG	TTCCAATCGG	540
GAAGCTTCGA	GTCAAGCTCC	ATTTATAGCC	AGCCTCTGCC	TAAAGTCCCC	CGGTAGATTG	600
GAGAGAATCT	TACTCAGAGC	TGCAAAATACC	CACAGTTCCG	CCAAACCTTG	CGGGCAACAA	660
TCCATTCACT	TGGGAGGAGT	ATTTGAATTG	CAACCAGGTG	CTTCGGTGTG	TGTCAATGTG	720
ACTGATCCAA	GCCAAGTGAG	CCATGGCACT	GGCTTCACGT	CCTTTGGCTT	ACTCAAATCT	780
TGA						783

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 558 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTGCTGCACT	TCGGGGTAAT	CGGCCCCCAG	AGGGAAGAGC	AGTCCCCAGG	TGGCCCCCTCC	60
ATCAACAGCC	CTCTGGTTCA	AACACTCAGG	TCCTCTTCTC	AAGCCTCAAG	TAACAAGCCG	120
GTAGCCACG	TTGTAGCCGA	CATCAACTCT	CCGGGGCAGC	TCCGGTGGTG	GGACTCGTAT	180
GCCAAATGCC	TCATGGCCAA	CGGTGTGAAG	CTGGAAGACA	ACCAGCTGGT	GGTGCCTGCT	240
GACGGGCTTT	ACCTCATCTA	CTCACAGGTC	CTCTTCAGGG	GCCAAGGCTG	CCCTTCCACC	300
CCCTTGTTC	TCACCCACAC	CATCAGCCGC	ATTGCAGTCT	CCTACCAGAC	CAAGGTCAAC	360
ATCCTGTCTG	CCATCAAGAG	CCCTTGCCAC	AGGGAGACCC	CAGAGTGGGC	TGAGGCCAAG	420
CCCTGGTACG	AACCCATCTA	CCAGGGAGGA	GTCTTCCAGC	TGGAGAAGGG	AGATCGCCTC	480
AGTGTCTGAGA	TCAACCTGCC	GGACTACCTG	GACTATGCCG	AGTCCGGGCA	GGTCTACTTT	540
GGGATCATTG	CCCTGTGA					558

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1783 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CAAGTCACAT	GATCCAGGAT	GCAGGGGAAA	ATCCTTCTTG	GAACAGAGCT	GGGTACAGAA	60
CCGAATCAGA	TGAGGAGAGA	TAAGGTGTGA	TGTGGGACAG	ACTATATAAA	GCATGGAGCC	120
AGGGCTGCAA	CAAGCAGGCA	GCTGTGGGGC	TCCTTCCCTT	GACCCAGCCA	TGCAGGTGCA	180
GCCCCGCTCG	GTAGCCAGCC	CCTGGAGAAG	CACGAGGCCC	TGGAGAAGCA	CAAGTCGCAG	240
CTACTTCTAC	CTCAGCACCA	CCGCACTGGT	GTGCCCTTGT	GTGGCAGTGG	CGATCATTCT	300
GGTACTGGTA	GTCCAGAAAA	AGGACTCCAC	TCCAAATACA	ACTGAGAAGG	CCCCCTTAA	360
AGGAGGAAAT	TGCTCAGAGG	ATCTCTTCTG	TACCCTGAAA	AGTACTCCAT	CCAAGAAGTC	420
ATGGGCCTAC	CTCCAAGTGT	CAAAGCATCT	CAACAATACC	AAACTGTCAT	GGAACGAAGA	480
TGGCACCATC	CACGGACTCA	TATACCAGGA	CGGGAACCTG	ATAGTCCAAT	TCCCTGGCTT	540
GTACTTCATC	GTTTGCCAAC	TGCAGTTCCT	CGTGCAGTGC	TCAAATCATT	CTGTGGACCT	600
GACATTGCAG	CTCCTCATCA	ATTCCAAGAT	CAAAAAGCAG	ACGTTGGTAA	CAGTGTGTGA	660
GTCTGGAGTT	CAGAGTAAGA	ACATCTACCA	GAATCTCTCT	CAGTTTTTGC	TGCATTACTT	720
ACAGGTCAAC	TCTACCATAT	CAGTCAGGGT	GGATAATTTT	CAGTATGTGG	ATACAAACAC	780
TTTCCCTCTT	GATAATGTGC	TATCCGTCTT	CTTATATAGT	AGCTCAGACT	GAATAGTTGT	840
TCTTAACCTT	TATGAAAAATG	CTGTCTACCA	TACAGTACTT	CATCTGTCCA	AACATGGGCC	900
AAAGAAAATA	TTAGGACAAC	TCAAACCTAAG	CATGTGAGTT	AGTGCACCTC	TCTTTCTGTC	960
CTTTGGAAAA	ATACAAACCC	AGGATTTAGA	AAGTGGAGTC	TCCTTCAGAT	GCACAAACAG	1020
GAAAGAATGT	GATATGTGCA	CAGAGACCTA	CTTGGGCACT	AGAAGGGGTG	TGAGTTGTCC	1080
CAGTATAACC	ACTAATTCAC	TGACCTTGAG	CCATTTTTTC	TTCCCCCTGG	AACTTGGGGT	1140
CTGAATCTGG	AAAAGTAGGA	GATGAGATTT	ACATTTCCCC	AATATTTTCT	TCAACTCAGA	1200
AGACGAGACT	GTGGAGCTGA	GCTCCCTACA	CAGATGAAGG	CCTCCCATGG	CATGAGGAAA	1260
ATGATGGTAC	CAGTAATGTC	TGTCTGACTG	TCATCTCAGC	AAGTCCTAAG	GACTTCCATG	1320
CTGCCTTGTT	GAAAGATACT	CTAACCTCTT	GTAATGGGCA	AAGTGATCCT	GTCTCTCACT	1380
GAGGGGAGTA	GCTGCTGCCA	TCTCCTGAGA	CATACATGGA	GACATTTTCT	GCCCCAATTC	1440
CATTCTGTGT	GCAGTTTTTA	AGTATTCCCC	CAAAAGTTCT	TGACAATGAG	AACTTTGAAT	1500
GTGGGAAGAG	CTTCTGGACA	GCAAACATTA	ACAGCTTCTC	CTGACCAGAG	AGACCATGCA	1560
AGCTTGGTCT	TAGACCCATC	AAGCTTGAGG	TTTCTACATT	GTGGGAGACA	GACTTTTGAC	1620
AAACCATTTG	AGTTGATGTC	TGGGCCCTTG	GGAGTTCTCC	TTCAAGTAAGG	AGAGCAAGCC	1680
GTTCTAGTGC	TGTGTCAGAG	GATGGAGTAA	AATAGACACT	TTTCTGAAGG	AAAGGAGAAC	1740
AAAGTTCCAG	AAAAAGGCTA	GAAATGTTT	AAAAAGAAAA	AAA		1783

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1047 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AGAGAGCGCT	GGGAGCCGGA	GGGGAGCGCA	GCGAGTTTGT	GCCAGTGGTC	GTGCAGTCCA	60
AGGGGCTGGA	TGGCATGCTG	GACCCAAGCT	CAGCTCAGCG	TCCGGACCCA	ATAACAGTTT	120
TACCAAGGGA	GCAGCTTTCT	ATCCTGGCCA	CACTGAGGTG	CATAGCGTAA	TGTCCATGTT	180
GTTCTACACT	CTGATCACAG	CTTTTCTGAT	CGGCATACAG	GCGGAACCA	ACTCAGAGAG	240
CAATGTCCCT	GCAGGACACA	CCATCCCCCA	AGTCCACTGG	ACTAAACTTC	AGCATTCCCT	300
TGACTGCGC	CTTCGCAGAG	CCCGCAGCGC	CCCGGCAGCG	GCGATAGCTG	CACGCGTGGC	360
GGGGCAGACC	CGCAACATTA	CTGTGGACCC	CAGGCTGTTT	AAAAAGCGGC	GACTCCGTTT	420
ACCCCGTGTG	CTGTTTAGCA	CCCAGCCTCC	CCGTGAAGCT	GCAGACACTC	AGGATCTGGA	480
CTTCGAGGTG	GGTGGTGTG	CCCCCTTCAA	CAGGACTCAC	AGGAGCAAGC	GGTCATCATC	540
CCATCCCATC	TTCACAGGG	GCGAATTCTC	GGTGTGTGAC	AGTGTGAGCG	TGTGGGTTGG	600
GGATAAGACC	ACCGCCACAG	ACATCAAGGG	CAAGGAGGTG	ATGGTGTGG	GAGAGGTGAA	660
CATTAAACAAC	AGTGTATTCA	AACAGTACTT	TTTTGAGACC	AAGTGCCGGG	ACCCAAATCC	720
CGTTGACAGC	GGGTGCCGGG	GCATTGACTC	AAAGCACTGG	AACTCATATT	GTACCACGAC	780
TCACACCTTT	GTCAAGCGCG	TGACCATGGA	TGGCAAGCAG	GCTGCCTGGC	GGTTTATCCG	840
GATAGATACG	GCCTGTGTGT	GTGTGCTCAG	CAGGAAGGCT	GTGAGAAGAG	CCTGACCTGC	900
CGACACGCTC	CTCCCCCTG	CCCCTTCTAC	ACTCTCCTGG	GCCCCCTCCT	ACCTCAACCT	960
GTAAATTATT	TTAAATTATA	AGGACTGCAT	GGTAATTTAT	AGTTTATACA	GTTTTAAAGA	1020
ATCATTATTT	ATTAAATTTT	TGGAAGC				1047

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1176 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GAGCGCCTGG	AGCCGGAGGG	GAGCGCATCG	AGTGACTTTG	GAGCTGGCCT	TATATTTGGA	60
TCTCCCGGGC	AGCTTTTITG	AAACTCCTAG	TGAACATGCT	GTGCCTCAAG	CCAGTGAAAT	120
TAGGCTCCCT	GGAGGTGGGA	CACGGGCAGC	ATGGTGGAGT	TTTGGCCTGT	GGTCGTGCAG	180
TCCAGGGGGC	TGGATGGCAT	GCTGGACCCA	AGCTCACCTC	AGTGTCTGGG	CCCAATAAAG	240
GTTTTGCCAA	GGACGCGAGT	TTCTATACTG	GCCGCAGTGA	GGTGCTAGC	GTAATGTCCA	300
TGTTGTCTTA	CACTCTGATC	ACTGCGTTTT	TGATCGGCGT	ACAGGCAGAA	CCGTACACAG	360
ATAGCAATGT	CCCAGAAGGA	GACTCTGTCC	CTGAAGCCCA	CTGGACTAAA	CTTCAGCATT	420
CCCTTGACAC	AGCCCTCCGC	AGAGCCCGCA	GTGCCCTTAC	TGCACCAATA	GCTGCCCGAG	480
TGACAGGGCA	GACCCGCAAC	ATCACTGTAG	ACCCGAGACT	GTTTAAAGAA	CGGAGACTCC	540
ACTCACCCCG	TGTGCTGTTT	AGCACCCAGC	CTCCACCCAC	CTCTTCAGAC	ACTCTGGATC	600
TAGACTTCCA	GGCCCATGGT	ACAATCCCTT	TCAACAGGAC	TCACCGGAGC	AAGCGCTCAT	660
CCACCCACCC	AGTCTTCCAC	ATGGGGGAGT	TCTCAGTGTG	TGACAGTGTG	AGTGTGTGGG	720
TTGGAGATAA	GACCACAGCC	ACAGACATCA	AGGGCAAGGA	GGTGACAGTG	CTGGCCGAGG	780
TGAACATTAA	CAACAGTGTA	TTCAGACAGT	ACTTTTGTGA	GACCAAGTGC	CGAGCCTCCA	840
ATCCTGTTGA	GAGTGGGTGC	CGGGGCATCG	ACTCAAACA	CTGGAACCTCA	TACTGCACCA	900
CGACTCACAC	CTTCGTCAAG	GCGTTGACAA	CAGATGAGAA	GCAGGCTGCC	TGGAGGTTCA	960
TCCGATAGA	CACAGCCTGT	GTGTGTGTGC	TCAGCAGGAA	GGCTACAAGA	AGAGGCTGAC	1020
TTGCCTGCAG	CCCCCTTCCC	CACCTGCCCC	CTCCACACTC	TCTTGGGCCC	CTCCCTACCT	1080
CAGCTGTAA	ATTATTTTAA	ATTATAAGGA	CTGCATGATA	ATTTATCGTT	TATACAATTT	1140
TAAAGACATT	ATTTATTAAA	TTTCAAAGC	ATCCTG			1176

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1623 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

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TCAGAGTCCT  GTCCTTGACA  CTTCAGTCTC  CACAAGACTG  AGAGGAGGAA  ACCCTTTCCT  60
GGGGCTGGGT  GCCATGCAGC  AGCCCGTGAA  TTACCCATGT  CCCCAGATCT  ACTGGGTAGA  120
CAGCAGTGCC  ACTTCTCCTT  GGGCTCCTCC  AGGGTCAGTT  TTTTCTTGTC  CATCCTCTGG  180
GCCTAGAGGG  CCAGGACAAA  GGAGACCACC  GCCTCCACCA  CCACCTCCAT  CACCACTACC  240
ACCGCCTTCC  CAACCACCCC  CGCTGCCTCC  ACTAAGCCCT  CTAAAGAAGA  AGGACAACAT  300
AGAGCTGTGG  CTACCGGTGA  TATTTTTCAT  GGTGCTGGTG  GCTCTGGTTG  GAATGGGGTT  360
AGGAATGTAT  CAACTCTTTC  ATCTACAGAA  GGAACCTGGC  GAACTCCGTG  AGTTCACCAA  420
CCACAGCCTT  AGAGTATCAT  CTTTGTAAAA  GCAAATAGCC  AACCCAGCA  CACCCTCTGA  480
AACCAGAAAG  CCAAGGAGTG  TGGCCCACTT  AACAGGGAAC  CCCCCTCAA  GGTCCATCCC  540
TCTGGAATGG  GAAGACACAT  ATGGAACCTG  TTTGATCTCT  GGAGTGAAGT  ATAAGAAAGG  600
CGGCCTTGTG  ATCAATGAGG  CTGGTTGTGA  CTTGCTATAT  TCCAAAGTAT  ACTTCCGGGG  660
TCAGTCTTGC  AACAGCCAGC  CCCTAAGCCA  CAAGGTCTAT  ATGAGGAACT  TTAAGTATCC  720
TGGGGATCTG  GTGCTAATGG  AGGAGAAGAA  GTTGAATTAC  TGCACTACTG  GCCAGATATG  780
GGCCACAGC  AGCTACCTAG  GGGCAGTATT  TAATCTTACC  GTTGCTGACC  ATTTATATGT  840
CAACATATCT  CAACTCTCTC  TGATCAATTT  TGAGGAATCT  AAGACCTTTT  TTGGCTTATA  900
TAAGCTTTAA  AGGAAAAGC  ATTTTAGAAT  GATCTATTAT  TCTTTATCAT  GGATGCCAGG  960
AATATGTGCT  TCAATGAGAG  TCTTCTTAAG  ACCAATTGAG  CCACAAAGAC  CACAAGGTCC  1020
AACAGGTCAG  CTACCCTTCA  TTTTCTAGAG  GTCCATGGAG  TGGTCTTAA  TGCCTGCATC  1080
ATGAGCCAGA  TGGGAAGAAG  ACTGTTCCCTG  AGGAACATAA  AGTTTTGGGC  TGCTGTGTGG  1140
CAATGCAGAG  GCAAAGAGAA  GGAACCTGCT  GATGTTAAAT  GGCCAAGAGC  ATTTAGCCA  1200
TTGAAGAAAA  AAAAAACCTT  TAACTCACC  TTCCAGGGTG  GGTCTACTTG  CTACCTCACA  1260
GGAGGCCGTC  TTTTAGACAC  ATGTTGTGTG  TATGACTATA  CAAGGGTGAG  AAAGGATGCT  1320
AGGTTTCATG  GATAAGCTAG  AGACTGAAAA  AAGCCAGTGT  CCCATTGGCA  TCATCTTTAT  1380
TTTTAACTGA  TGTTTTCTGA  GCCACCTTT  GATGCTAACA  GAGAAATAAG  AGGGGTGTTT  1440
GAGGCACAAG  TCATTCTCTA  CATAGCATGT  GTACCTCCAG  TGCAATGATG  TCTGTGTGTG  1500
TTTTTATGTA  TGAGAGTAGA  GCGATTCTAA  AGAGTCACAT  GAGTACAACG  CGTACATTAC  1560
GGAGTACATA  TTAGAAACGT  ATGTGTTACA  TTTGATGCTA  GAATATCTGA  ATGTTTCTTG  1620
CTA

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(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GTTAAGCTTT TCAGTCAGCA TGATAGAA

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(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GTTTCTAGAT CAGAGTTTGA GTAAGCC

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(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CCAAGACTAG TTAACACAGC ATGATCGAAA

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(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CCAATGCCGC CGCACTCAGA ATTCAACCTG

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(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 972 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TCTAGACTCA	GGACTGAGAA	GAAGTAAAC	CGTTTGCTGG	GGCTGGCCTG	ACTCACCAGC	60
TGCCATGCAG	CAGCCCTTCA	ATTACCCATA	TCCCCAGATC	TACTGGGTGG	ACAGCAGTGC	120
CAGCTCTCCC	TGGGCCCCCTC	CAGGCACAGT	TCTTCCCTGT	CCAACCTCTG	TGCCCAGAAG	180
GCCTGGTCAA	AGGAGGCCAC	CACCACCACC	GCCACCGCCA	CCACTACCAC	CTCCGCCGCC	240
GCCGCCACCA	CTGCCTCCAC	TACCGCTGCC	ACCCCTGAAG	AAGAGAGGGA	ACCACAGCAC	300
AGGCCTGTGT	CTCCTTGTGA	TGTTTTTCAT	GGTCTGGTT	GCCTTGGTAG	GATTGGGCCT	360
GGGGATGTTT	CAGCTCTTCC	ACCTACAGAA	GGAGCTGGCA	GAACCTCGAG	AGTCTACCAG	420
CCAGATGCAC	ACAGCATCAT	CTTTGGAGAA	GCAAATAGGC	CACCCAGTC	CACCCCTGA	480
AAAAAAGGAG	CTGAGGAAAG	TGGCCCATTT	AACAGGCAAG	TCCAACCTCA	GGTCCATGCC	540
TCTGGAATGG	GAAGACACCT	ATGGAATTGT	CCTGCTTTCT	GGAGTGAAGT	ATAAGAAGGG	600
TGGCCTTGTT	ATCAATGAAA	CTGGGCTGTA	CTTTGTATAT	TCCAAAGTAT	ACTTCCGGGG	660
TCAATCTTGC	AACAACCTGC	CCCTGAGCCA	CAAGGTCTAC	ATGAGGAACT	CTAAGTATCC	720
CCAGGATCTG	GTGATGATGG	AGGGGAAGAT	GATGAGCTAC	TGCACTACTG	GGCAGATGTG	780
GGCCCGCAGC	AGCTACCTGG	GGGCAGTGTT	CAATCTTACC	AGTGCTGATC	ATTTATATGT	840
CAACGTATCT	GAGCTCTCTC	TGGTCAATTT	TGAGGAATCT	CAGACGTTTT	TCGGCTTATA	900
TAAGCTCTAA	GAGAAGCACT	TTGGGATTCT	TTCCATTATG	ATTCTTTGTT	ACAGGCACCG	960
AGATGTTCTA	GA					972

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 885 base pairs

121

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATGCAGCAGC	CCATGAATTA	CCCATGTCCC	CAGATCTTCT	GGGTAGACAG	CAGTGCCACT	60
TCATCTTGGG	CTCCTCCAGG	GTCACTTTT	CCCTGTCCAT	CTGTGCGGC	TAGAGGGCCG	120
GACCAAAGGA	GACCGCCACC	TCCACCACCA	CCTGTGTCAC	CACTACCACC	GCCATCACAA	180
CCACTCCAC	TGCGGCCACT	GACCCCTCTA	AAGAAGAAGG	ACCACAACAC	AAATCTGTGG	240
CTACCGGTGG	TATTTTTCAT	GGTCTGGTG	GCTCTGGTG	GAATGGGATT	AGGAATGTAT	300
CAGCTCTTCC	ACCTGCAGAA	GGAAGTGGCA	GAAGTCCGTG	AGTTCACCAA	CCAAAGCCTT	360
AAAGTATCAT	CTTTTGAAAA	GCAATAGCC	AACCCAGTA	CACCCCTCTGA	AAAAAAGAG	420
CCGAGGAGTG	TGGCCCATTT	AACAGGGAAC	CCCCACTCAA	GGTCCATCCC	TCTGGAATGG	480
GAAGACACAT	ATGGAACCGC	TCTGATCTCT	GGAGTGAAGT	ATAAGAAAGG	TGGCCTTGTG	540
ATCAACGAAG	CTGGGTTGTA	CTTCGTATAT	TCCAAAGTAT	ACTTCCGGGG	TCAGTCTTGC	600
AACAACCAGC	CCCTAAACCA	CAAGGTCTAT	ATGAGGAACT	CTAAGTATCC	TGGGGATCTG	660
GTGCTAATGG	AGGAGAAGAG	GTTGAACTAC	TGCACTACTG	GACAGATATG	GGCCACAGC	720
AGCTACCTGG	GGGCAGTATT	CAATCTTACC	AGTGCTGACC	ATTTATATGT	CAACATATCT	780
CAACTCTCTC	TGATCAATTT	TGAGGAATCT	AAGACCTTTT	TCGGCTTGTA	TAAGCTTTAA	840
AAGAAAAAGC	ATTTTAAAT	GATCTACTAT	TCTTTATCAT	GGGCA		885

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CTTAAGCTTC TACAGGACTG AGAAGAAGT

29

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CTTGAATTCC AACATTCTCG GTGCCTGTAA

27

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

TCAGGATCCA CAAGGCTGTG AGAAGGA

27

122

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CTTGTCTAGA CCTGGTGCC CATGATA

27

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 680 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

ATGCCGGAGG	AAGGTCGCCC	TTGCCCCCTGG	GTTCCGCTGGA	GCGGGACCGC	GTTCCAGCGC	60
CAATGGCCAT	GGCTGCTGCT	GGTGGTGT	TTTACTGTGT	TTTGCTGTG	GTTTCATTGT	120
AGCGGACTAC	TCAGTAAGCA	GCAACAGAGG	CTGCTGGAGC	ACCCTGAGCC	GCACACAGCT	180
GAGTTACAGC	TGAATCTCAC	AGTTCCTCGG	AAGGACCCCA	CACTGCGCTG	GGGAGCAGGC	240
CCAGCCTTGG	GAAGGTCCTT	CACACACGGA	CCAGAGCTGG	AGGAGGGCCA	TCTGCGTATC	300
CATCAAGATG	GCCTCTACAG	GCTGCATATC	CAGGTGACAC	TGGCCAACTG	CTCTTCCCCA	360
GGCAGCACCC	TGCAGCACAG	GGCCACCCTG	GCTGTGGGCA	TCTGCTCCCC	CGCTGCGCAC	420
GGCATCAGCT	TGCTGCGTGG	GCGCTTTGGA	CAGGACTGTA	CAGTGGCATT	ACAGCGCCTG	480
ACATACCTGG	TCCACGGAGA	TGTCTCTGT	ACCAACCTCA	CCCTGCCTCT	GCTGCGCTCC	540
CGCAACGCTG	ATGAGACCTT	CTTTGGAGTT	CAGTGGATAT	GCCCTTGACC	ACAACCTCCAG	600
GATGACTTGT	GAATATTTT	TTTCTTTTCA	AGTTCTACGT	ATTATATAAT	GTATATAGTA	660
CACATAAAAA	AAAAAAAAAA					680

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 846 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CCTCTGTGCC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCACTGC	CTCCACTACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240
CTGTGTCTCC	TTGTGATGTT	TTTCATGGTT	CTGGTTGCCT	TGGTAGGATT	GGGCCTGGGG	300
ATGTTTCAGC	TCTTCCACCT	GCAGAAGGAA	CTGGCAGAAC	TCCGTGAGTT	CACCAACCAA	360
AGCCTTAAAG	TATCATCTTT	TGAAAAGCAA	ATAGGCCACC	CCAGTCCACC	CCCTGAAAAA	420
AAGGAGCTGA	GGAAAGTGCC	CCATTTAACA	GGCAAGTCCA	ACTCAAGGTC	CATGCCTCTG	480
GAATGGGAAG	ACACCTATGG	AATTGTCCTG	CTTCTGGAG	TGAAGTATAA	GAAGGGTGCC	540
CTGTGATCA	ATGAAACTGG	GCTGTACTTT	GTATATTCCA	AAGTATACTT	CCGGGGTCAA	600
TCTTGCAACA	ACCTGCCCCT	GAGCCACAAG	GTCTACATGA	GGAACCTCTAA	GTATCCCCAG	660
GATCTGGTGA	TGATGGAGGG	GAAGATGATG	AGCTACTGCA	CTACTGGGCA	GATGTGGGCC	720
CCGAGCAGCT	ACCTGGGGGC	AGTGTTCAT	CTTACCAGTG	CTGATCATT	ATATGTCAAC	780

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GTATCTGAGC TCTCTCTGGT CAATTTTGAG GAATCTCAGA CGTTTTTTCGG CTTATATAAG
CTCTAA

840
846

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 786 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CCTCTGTGCC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CCGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCACTGC	CTCCACTACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240
CTGTGTCTCC	TTGTGATGTT	TTTCATGGTT	CTGGTTGCCT	TGGTAGGATT	GGGCCTGGGG	300
ATGTTTCAGC	TCTTCCGCTT	CGCACAGGCT	ATAGGCCACC	CCAGTCCACC	CCCTGAAAAA	360
AAGGAGCTGA	GGAAAGTGGC	CCATTTAACA	GGCAAGTCCA	ACTCAAGGTC	CATGCCTCTG	420
GAATGGGAAG	ACACCTATGG	AATTGTCTCG	CTTTCTGGAG	TGAAGTATAA	GAAGGGTGGC	480
CTTGTGATCA	ATGAAACTGG	GCTGTACTTT	GTATATTCCA	AAGTATACTT	CCGGGGTCAA	540
TCTTGCAACA	ACCTGCCCTT	GAGCCACAAG	GTCTACATGA	GGAACCTCTA	GTATCCCCAG	600
GATCTGGTGA	TGATGGAGGG	GAAGATGATG	AGCTACTGCA	CTACTGGGCA	GATGTGGGCC	660
CGCAGCAGCT	ACCTGGGGGC	AGTGTTCAT	CTTACCAGTG	CTGATCATTT	ATATGTCAAC	720
GTATCTGAGC	TCTCTCTGGT	CAATTTTGAG	GAATCTCAGA	CGTTTTTTCGG	CTTATATAAG	780
CTCTAA						786

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 864 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CCTCTGTGCC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CCGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCACTGC	CTCCACTACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240
CTGTGTCTCC	TTGTGATGTT	TTTCATGGTT	CTGGTTGCCT	TGGTAGGATT	GGGCCTGGGG	300
ATGTTTCAGC	TCTTCCAATC	CTCCATCCTC	CCCTATGCCG	GAGGAGGGTT	CGGGCTGCTC	360
GGTGCGGGCG	AGGCCCTATG	GGTGCGTCCT	GCGGCCATCC	TCAATCCTAT	AGGCCACCCC	420
AGTCCACCCC	CTGAAAAAAA	GGAGCTGAGG	AAAGTGGCCC	ATTTAACAGG	CAAGTCCAAC	480
TCAAGGTCCA	TGCCTCTGGA	ATGGGAAGAC	ACCTATGGAA	TTGTCTTGCT	TTCTGGAGTG	540
AAGTATAAGA	AGGGTGGCCT	TGTGATCAAT	GAAACTGGGC	TGTACTTTGT	ATATTCCAAA	600
GTATACTTCC	GGGGTCAATC	TTGCAACAAC	CTGCCCCGTA	GCCACAAGGT	CTACATGAGG	660
AATCTAAGT	ATCCCCAGGA	TCTGGTGATG	ATGGAGGGGA	AGATGATGAG	CTACTGCACT	720
ACTGGGCAGA	TGTGGGCCCG	CAGCAGCTAC	CTGGGGGCAG	TGTTCAATCT	TACCAAGTGT	780
GATCATTTAT	ATGTCAACGT	ATCTGAGCTC	TCTCTGGTCA	ATTTTGAGGA	ATCTCAGACG	840
TTTTTCGGCT	TATATAAGCT	CTAA				864

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 828 base pairs

124

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CCTCTGTGCC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CCGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCACTGC	CTCCACTACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240
CTGTGTCTCC	TGTGTATGTT	TTTCATGGTT	CTGGTTGCCT	TGGTAGGATT	GGGCCTGGGG	300
ATGTTTCAGC	TCTTCCACCT	ACAGCGAGAG	TCTACCAGCC	AGATGCACAC	AGCATCATCT	360
TTGGAGAAGC	AAATAGGCCA	CCCCAGTCCA	CCCCCTGAAA	AAAAGGAGCT	GAGGAAAGTG	420
GCCCATTTAA	CAGGCAAGTC	CAACTCAAGG	TCCATGCCTC	TGGAATGGGA	AGACACCTAT	480
GGAATTGTCC	TGCTTTCTGG	AGTGAAGTAT	AAGAAGGGTG	GCCTTGTGAT	CAATGAAACT	540
GGGCTGTACT	TTGTATATTC	CAAAGTATAC	TTCCGGGGTC	AATCTTGCAA	CAACCTGCCC	600
CTGAGCCACA	AGGTCTACAT	GAGGAACTCT	AAGTATCCCC	AGGATCTGGT	GATGATGGAG	660
GGGAAGATGA	TGAGCTACTG	CACTACTGGG	CAGATGTGGG	CCCGCAGCAG	CTACCTGGGG	720
GCAGTGTTC	ATCTTACCAG	TGCTGATCAT	TTATATGTC	ACGTATCTGA	GCTCTCTCTG	780
GTCAATTTTG	AGGAATCTCA	GACGTTTTTC	GGCTTATATA	AGCTCTAA		828

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 846 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

ATGGCTATGA	TGGAGGTCCA	GGGGGGACCC	AGCCTGGGAC	AGACCTGCGT	GCTGATCGTG	60
ATCTTCACAG	TGCTCCTGCA	GTCTCTCTGT	GTGGCTGTAA	CTTACGTGTA	CTTTACCAAC	120
GAGCTGAAGC	AGATGCAGGA	CAAGTACTCC	AAAAGTGCCA	TTGCTTGTTC	CTTAAAAGAA	180
GATGACAGTT	ATTGGGACCC	CAATGACGAA	GAGAGTATGA	ACAGCCCCTG	CTGGCAAGTC	240
AAGTGGCAAC	TCCGTCAGCT	CGTTAGAAAG	ATGATTTTGA	GAACCTCTGA	GGAAACCATT	300
TCTACAGTTC	AAGAAAAGCA	ACAAAATATT	TCTCCCTTAG	TGAGAGAAAG	AGGTCCTCAG	360
AGAGTAGCAG	CTCACATAAC	TGGGACCAGA	GGAAGAAGCA	ACACATTGTC	TTCTCCAAAC	420
TCCAAGAATG	AAAAGGCTCT	GGGCCGCAAA	ATAAACTCCT	GGGAATCATC	AAGGAGTGCG	480
CATTCACTTC	TGAGCAACTT	GCACTTGAGG	AATGGTGAAC	TGGTCATCCA	TGAAAAGGGG	540
TTTTACTACA	TCTATTCCCA	AACATACTTT	CGATTTCAGG	AGGAAATAAA	AGAAAACACA	600
AAGAACGACA	AACAAATGGT	CCAATATATT	TACAAATACA	CAAGTTATCC	TGACCCTATA	660
TTGTTGATGA	AAAGTGCTAG	AAATAGTTGT	TGGTCTAAAG	ATGCAGAATA	TGGACTCTAT	720
TCCATCTATC	AAGGGGGAAT	ATTGAGCTT	AAGGAAAATG	ACAGAAATTT	TGTTTCTGTA	780
ACAAATGAGC	ACTTGATAGA	CATGGACCAT	GAAGCCAGTT	TTTTCGGGGC	CTTTTGTAGT	840
GGCTAA						846

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 876 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

ATGCCCTTCT	CAGGGGCCCT	GAAGGACCTC	AGCTTCAGTC	AGCACTTCAG	GATGATGGTG	60
ATTGTCATAG	TGCTCCTGCA	GGTGCTCCTG	CAGGCTGTGT	CTGTGGCTGT	GACTTACATG	120

125

TACTTCACCA	ACGAGATGAA	GCAGCTGCAG	GACAATTACT	CCAAAATTGG	ACTAGCTTGC	180
TTCTCAAAGA	CGGATGAGGA	TTTCTGGGAC	TCCACTGATG	GAGAGATCTT	GAACAGACCC	240
TGCTTGCAAG	TTAAGAGGCA	ACTGTATCAG	CTCATTGAAG	AGGTGACTTT	GAGAACCTTT	300
CAGGACACCA	TTTCTACAGT	TCCAGAAAAG	CAGCTAAGTA	CTCCTCCCTT	GCCCAGAGGT	360
GGAAGACCTC	AGAAAGTGGC	AGCTCACATT	ACTGGGATCA	CTCGGAGAAG	CAACTCAGCT	420
TTAATTCCAA	TCTCCAAGGA	TGGAAAGACC	TTAGGCCAGA	AGATTGAATC	CTGGGAGTCC	480
TCTCGGAAAG	GGCATTTCATT	TCTCAACCAC	GTGCTCTTTA	GGAATGGAGA	GCTGGTCATC	540
GAGCAGGAGG	GCCTGTATTA	CATCTATTCC	CAAACATACT	TCCGATTTC	GGAAGCTGAA	600
GACGCTTCCA	AGATGGTCTC	AAAGGACAAG	GTGAGAACCA	AACAGCTGGT	GCAGTACATC	660
TACAAGTACA	CCAGCTATCC	GGATCCCAT	GTGCTCATGA	AGAGCGCCAG	AAACAGCTGT	720
TGGTCCAGAG	ATGCCGAGTA	CGGACTGTAC	TCCATCTATC	AGGGAGGATT	GTTCCAGCTA	780
AAAAAAATG	ACAGGATTTT	TGTTCTGTG	ACAAATGAAC	ATTTGATGGA	CCTGGATCAA	840
GAAGCCAGCT	TCTTTGGAGC	CTTTTAATT	AATAA			876

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	720 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

ATGGAGCCAG	GGCTGCAACA	AGCAGGCAGC	TGTGGGGCTC	CTTCCCCTGA	CCCAGCCATG	60
CAGGTGCAGC	CCGGCTCGGT	AGCCAGCCCC	TGGAGAAGCA	CGAGGCCCTG	GAGAAGCACA	120
AGTCGCAGCT	ACTTCTACCT	CAGCACCACC	GCACTGGTGT	GCCTTGTTGT	GGCAGTGGCG	180
ATCATTCTGG	TACTGGTAGT	CCAGAAAAAG	GACTCCACTC	CAAATACAAC	TGAGAAGGCC	240
CCCCTTAAAG	GAGGAAATTG	CTCAGAGGAT	CTCTTCTGTA	CCCTGAAAAG	TACTCCATCC	300
AAGAAGTCAT	GGGCTTACCT	CCAAGTGTC	AAGCATCTCA	ACAATACCAA	ACTGTCATGG	360
AACGAAGATG	GCACCATCCA	CGGACTCATA	TACCAGGACG	GGAACCTGAT	AGTCCAATTC	420
CCTGGCTTGT	ACTTCATCGT	TTGCCAACTG	CAGTTCCTCG	TGCAGTGTCT	AAATCATTCT	480
GTGGACCTGA	CATTGCAGCT	CCTCATCAAT	TCCAAGATCA	AAAAGCAGAC	GTTGGTAACA	540
GTGTGTGAGT	CTGGAGTTCA	GAGTAAGAAC	ATCTACCAGA	ATCTCTCTCA	GTTTTTGCTG	600
CATTACTTAC	AGGTCAACTC	TACCATATCA	GTCAGGGTGG	ATAATTTCCA	GTATGTGGAT	660
ACAAACACTT	TCCCTCTTGA	TAATGTGCTA	TCCGTCTTCT	TATATAGTAG	CTCAGACTGA	720

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	930 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

ATGGACCAGC	ACACACTTGA	TGTGGAGGAT	ACCGCGGATG	CCAGACATCC	AGCAGGTACT	60
TCGTGCCCTT	CGGATGCGGC	GCTCCTCAGA	GATACCGGGC	TCCTCGCGGA	CGCTGCGCTC	120
CTCTCAGATA	CTGTGCGCCC	CACAAATGCC	GCGCTCCCCA	CGGATGTGTC	CTACCCTGCG	180
GTTAATGTTC	GGGATCGCGA	GGCCGCGTGG	CCGCTTGAC	TGAACCTTCTG	TTCCCGCCAC	240
CCAAAGCTCT	ATGGCCTAGT	CGCTTGGTT	TGCTGCTTC	TGATCGCCGC	CTGTGTTCTT	300
ATCTTCAACC	GCACCGAGCC	TCGGCCAGCG	CTCACAATCA	CCACCTCGCC	CAACCTGGGT	360
ACCCGAGAGA	ATAATGCAGA	CCAGGTCACC	CCTGTTTCCC	ACATTGGCTG	CCCCAACACT	420
ACACAACAGG	GCTCTCCTGT	GTTCCGCAAG	CTACTGGCTA	AAAACCAAGC	ATCGTGTGTC	480
AATACAACCT	TGAAGTGGCA	CAGCCAAGAT	GGAGCTGGGA	GCTCATACCT	ATCTCAAGGT	540
CTGAGGTACG	AAGAAGACAA	AAAGGAGTTG	GTGGTAGACA	GTCCCGGGCT	CTACTACGTA	600
TTTTTTGGAAC	TGAAGCTCAG	TCCAACATTC	ACAAACACAG	GCCACAAGGT	GCAGGGCTGG	660
GTCTCTCTTG	TTTTGCAAGC	AAAGCTCAG	GTAGATGACT	TTGACAACCT	GGCCCTGACA	720
GTGGAACGTG	TCCCTTGCTC	CATGGAGAAC	AAGTTAGTGG	ACCGTTCCCTG	GAGTCAACTG	780
TTGCTCTGTA	AGGCTGGCCA	CCGCTCAGT	GTGGGTCTGA	GGGCTTATCT	GCATGGAGCC	840
CAGGATGCAT	ACAGAGACTG	GGAGCTGTCT	TATCCCAACA	CCACCAGCTT	TGGACTCTTT	900
CTTGTGAAAC	CCGACAACCC	ATGGAATGA				930

Claims

1. A method of altering the immunoreactivity of human cells, which method comprising introducing a gene
5 encoding an accessory molecule ligand into said cells so that said accessory molecule ligand is expressed on the surface of said cells.
2. The method of claim 1 wherein the accessory
10 molecule to which the accessory molecule ligand specifically binds is also present on the surface of said human cells.
3. The method of claim 1 wherein said human cells
15 are neoplastic human cells.
4. The method of claim 1 wherein said accessory molecule ligand gene is a chimeric gene.
- 20 5. The method in claim 1 wherein said accessory molecule ligand gene is present in a vector capable of transducing human cells.
6. The method of claim 1 wherein said accessory
25 molecule ligand gene is present as part of a genetic vector.
7. The method of claim 1 wherein said accessory molecule ligand gene is operatively linked to a promoter
30 region and a polyadenylation signal.
8. The method of claim 1 wherein said gene is a CD40 ligand gene.
- 35 9. The method of claim 7 wherein said CD40 ligand gene is a murine CD40 ligand gene.

10. The method of claim 4 wherein said gene is a chimeric gene which comprises at least a portion of a murine CD40 ligand gene.

5 11. A method of treating a human neoplasia comprising inserting into said human neoplastic cells a gene which encodes an accessory molecule ligand into said human neoplastic cells so that said accessory molecule ligand is expressed on the surface of said neoplastic
10 cells.

12. The method of claim 11 further comprising:
a) obtaining said human neoplastic cells from a human patient;
15 b) infusing said human neoplastic cells after having inserted said accessory molecule ligand on the surface of said cells back into said patient.

13. The method of claim 11 wherein the accessory
20 molecule to which the said accessory molecule ligand specifically binds is present on the surface of said human neoplastic cells.

14. The method of claim 11 wherein said accessory
25 molecule ligand gene is a chimeric gene.

15. The method of claim 11 wherein said accessory molecule ligand gene is a chimeric gene which contains at least a portion of the murine CD40 ligand gene.
30

16. The method of claim 11 wherein said accessory molecule ligand gene is present as part of a genetic vector.

35 17. The method of claim 11 wherein said accessory molecule ligand gene is operatively linked to a promoter region and a 3' end region.

18. The method of claim 11 wherein said accessory molecule ligand gene is a CD40 ligand gene.

19. The method of claim 11 wherein the said CD40
5 ligand gene is a murine CD40 ligand gene.

20. The method of claim 11 wherein said accessory molecule ligand gene is a Fas-ligand gene or a CD27
ligand gene.

10

21. The method of claim 11 wherein said accessory molecule ligand gene is present in a gene therapy vector.

22. A method of treating a neoplasia in a patient
15 comprising injecting into the tumor bed of said patient a gene which encodes an accessory molecule ligand so that said accessory molecule ligand is expressed on the surface of said tumor cells thereby causing said cells to participate in an immune reaction.

20

23. A gene therapy vector containing an accessory molecule ligand gene.

24. The gene therapy vector of claim 23 wherein
25 said accessory molecule gene is a CD40 ligand gene.

25. The gene therapy vector of claim 23 wherein said CD40 ligand gene is a murine CD40 ligand gene.

26. The gene therapy vector of claim 23 wherein
30 said CD40 ligand gene is a chimeric gene.

27. The gene therapy vector of claim 23 wherein said chimeric gene contains at least a portion of the
35 murine CD40 ligand gene.

28. The gene therapy vector of claim 23 wherein at least a portion of said vector is derived from adenovirus DNA.

5 29. The gene therapy vector of claim 23 wherein said vector contains at least a promoter region and a 3' end region.

10 30. The gene therapy vector of claim 23 wherein said promoter region and said 3' end region are not derived from the same species from which the CD40 ligand gene is derived.

15 31. The gene therapy vector of claim 23 wherein said portion of said vector is derived from viral DNA.

32. The gene therapy vector of claim 23 wherein at least a portion of said vector is derived from a retrovirus.

20

33. The gene therapy vector of claim 23 wherein said vector is capable of transducing human cells.

25 34. The gene therapy vector of claim 23 wherein said vector is capable of transducing animal cells.

35. The gene therapy vector of claim 23 wherein said human cells are human neoplastic cells.

30 36. The gene therapy vector of claim 23 wherein said human cells are human antigen presenting cells.

37. A genetic construct containing a promoter operatively linked to an accessory molecule ligand gene
35 which is also operatively linked to a polyadenylation signal.

38. A genetic construct in which a promoter is operatively linked to a chimeric accessory molecule ligand gene and a polyadenylation signal.

5 39. A gene therapy vector containing a chimeric accessory molecule ligand gene.

40. The gene therapy vector of claim 39 wherein said chimeric accessory molecule ligand gene contains at
10 least one gene segment derived from a murine CD40 ligand gene and other gene segments derived from other accessory molecule genes.

41. The gene therapy vector of claim 39 wherein
15 said other accessory molecule ligand genes are human accessory molecule ligand genes.

42. The gene therapy vector of claim 39 wherein said human accessory molecule ligand genes are human CD40
20 ligand genes.

43. A human cell containing the gene therapy vector of claims 23-36 or 39-42 or the genetic construct of claims 37-38.

25

44. The human cell of claim 43 wherein said cell is an antigen presenting cell.

45. The human cell of claim 43 wherein said human
30 cell is a neoplastic cell.

46. The human cell of claim 43 wherein said cell is an accessory cell.

35 47. An animal cell containing the gene therapy vector or genetic construct of claims 23-43.

48. An insect cell containing the gene therapy vector or genetic construct of claims 23-43.

49. A bacterial cell containing the gene therapy
5 vector or genetic construct of claims 23-43.

50. A method of vaccinating an animal against a predetermined organism comprising: administering into an animal to be immunized against a predetermined organism,
10 a vaccine comprising immunogenic antigens capable of causing an immune response to said predetermined organism together with a vector containing a gene including an accessory molecule ligand.

15 51. The method of claim 50 wherein said immunogenic antigens are encoded by genes present on a genetic vector.

52. The method of claim 50 wherein said gene is a
20 chimeric gene.

53. The method of claim 50 wherein said chimeric gene contains at least a portion of a murine CD40 ligand gene.

25

54. The method of claim 50 wherein said chimeric gene contains at least a segment of a murine CD40 ligand gene and at least a segment of a different accessory molecule gene.

30

55. The method of claim 50 wherein said predetermined organism is a virus, a bacteria, a fungus or a neoplastic cell.

35 56. A method of producing an immune response directed to a predetermined antigen comprising: administering to said animal a genetic vector containing

a gene encoding the antigen to which said immune response is desired together with a genetic vector containing a gene encoding an accessory molecule ligand gene.

5 57. A chimeric accessory molecule ligand gene comprising at least one domain or sub-domain gene segment derived from a first accessory molecule ligand gene operatively linked to the domain or sub-domain gene segment of a second accessory molecule ligand gene.

10

58. The chimeric accessory molecule ligand gene of claim 57 in which said first and second accessory molecule ligand genes are selected from the group consisting of the genes from any species encoding a member of the tumor necrosis family, CD40-ligand, Fas-ligand, CD70, TNF α , TNF β , CD30 ligand, 4-1BB ligand (4-1BBL), TNF-related apoptosis inducing ligand (TRAIL) and nerve growth factor.

20 59. The chimeric accessory molecule ligand gene of claim 57 in which at least one of said domain or sub-domain gene segments is an artificial gene segment.

60. The chimeric accessory molecule encoded by the
25 genes of claims 57-59.

61. A chimeric accessory molecule ligand gene comprising at least a portion of the gene encoding Domains I and II derived from an accessory molecule ligand operatively linked to at least a portion of the
30 gene encoding a Domain of an accessory molecule ligand which in turn is operatively linked to at least a portion of the gene encoding Domain IV of an accessory molecule ligand.

35

62. The chimeric accessory molecule ligand gene of claim 61 wherein said Domains I and II are derived from the human CD40 ligand gene.

5 63. The chimeric accessory molecule ligand gene of claim 61 wherein said Domain IV is the human Fas-ligand Domain IV.

10 64. The chimeric accessory molecule ligand gene of claim 61 wherein said Domain is Domain III of another accessory molecule ligand.

15 65. The chimeric accessory molecule ligand gene of claim 61 wherein said Domain is a domain from the same accessory molecule ligand.

66. The chimeric accessory molecule ligand gene of claim 61 wherein said Domain is an artificial domain.

20 67. The method of claim 4 wherein said chimeric gene is a gene of claims 57-60.

25 68. The method of claim 11 wherein said accessory molecule ligand gene is a chimeric accessory molecule ligand gene of claims 57-60.

30 69. The gene therapy vector of claim 23 wherein said chimeric gene is a chimeric accessory molecule ligand gene of claims 57-60.

70. A method of treating rheumatoid arthritis in a joint comprising inserting into the joint a vector containing a gene which encodes an accessory molecule ligand so that said accessory molecule ligand is
35 expressed on the surface of cells within the joint.

71. The method of claim 70 wherein said accessory molecule ligand gene is a chimeric accessory molecule ligand gene which is comprised of at least a portion of a human Fas-ligand gene.

5

72. The method of claim 70 wherein said accessory molecule ligand gene is a chimeric accessory molecule ligand gene which contains at least a portion of the murine Fas-ligand gene.

10

73. The method of claim 70 wherein said accessory molecule ligand gene is a murine Fas-ligand gene.

74. The method of claim 70 wherein said accessory molecule ligand gene is the murine Fas-ligand gene.

75. The method of claim 70 wherein said accessory molecule ligand gene is a chimeric accessory molecule gene comprised of at least a portion of domain III from the murine Fas-ligand gene and a portion of domain IV from the human Fas-ligand gene.

76. The method of claim 70 wherein said accessory molecule ligand gene is a chimeric accessory molecule ligand gene comprised of a portion of domain III of the human CD70 gene and at least a portion of domain IV of the human Fas-ligand gene.

77. A method of treating rheumatoid arthritis in a joint comprising inserting into the joint cells which have been transformed with a gene which encodes on accessory molecule ligand which is expressed on the surface of said cells.

78. A chimeric accessory molecule ligand comprised of at least a portion of the fourth domain of human Fas-ligand.

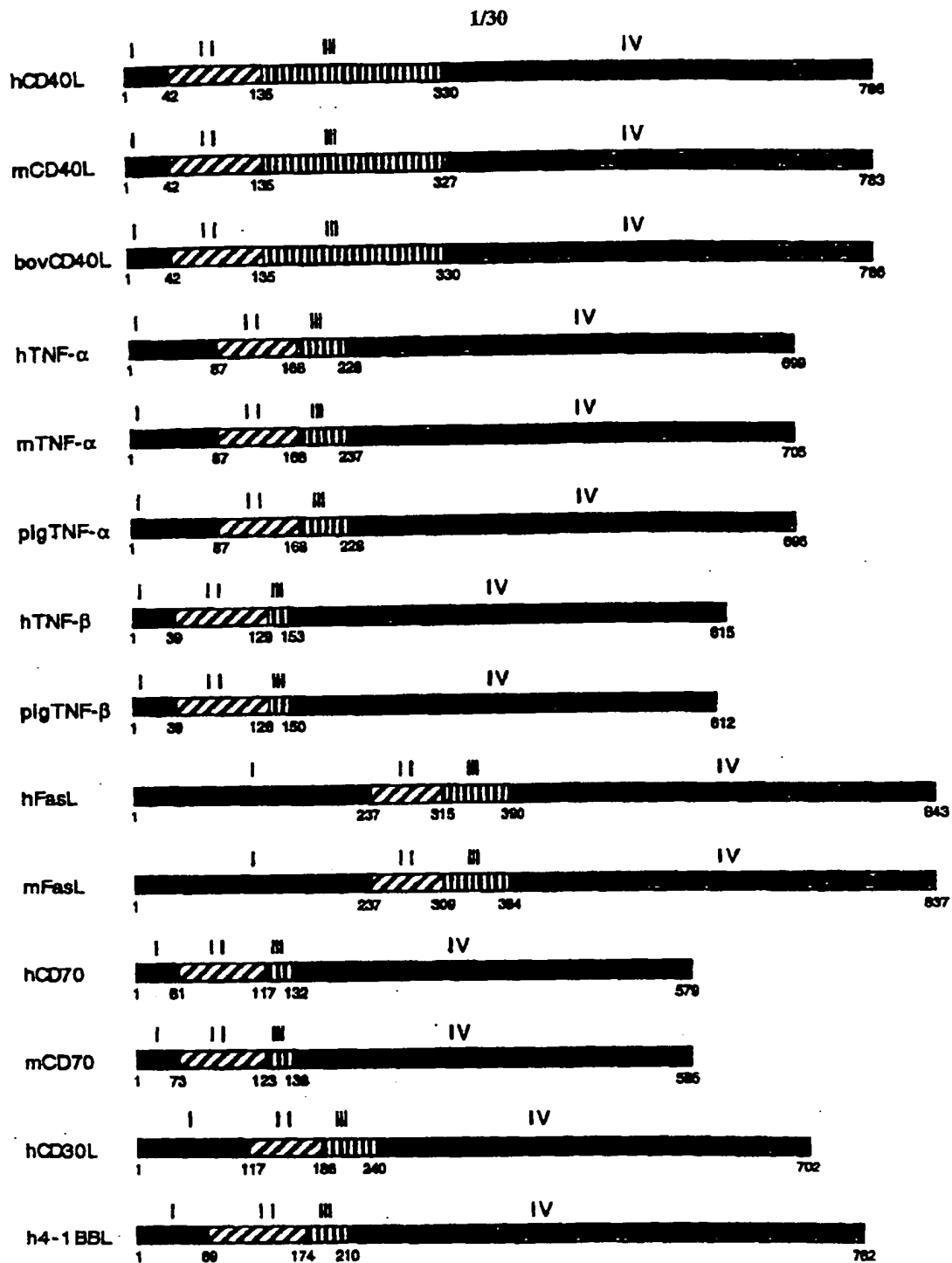
79. A chimeric accessory molecule ligand derived from a Fas-ligand in which at least one matrix metalloproteinase cleavage site has been removed.

5 80. A chimeric accessory molecule ligand comprised of domain III of the Murine Fas-ligand or the human CD70 gene, and domain IV of the human Fas-ligand.

81. A gene therapy vector containing a gene
10 encoding chimeric accessory molecule of claims 78-80.

82. A cell containing a gene therapy vector of claim 81.

15 83. A method of altering the immunoreactivity of human cells, which method comprising introducing a gene encoding an accessory molecule ligand which has a stabilized activity into said cells so that said accessory molecule ligand is expressed on the surface of
20 said cells.



DOMAINS: I - Cytoplasmic Domain; II - Transmembrane Domain; III - Proximal Extracellular Domain; IV - Distal Extracellular Domain (putative soluble form)

Figure 1

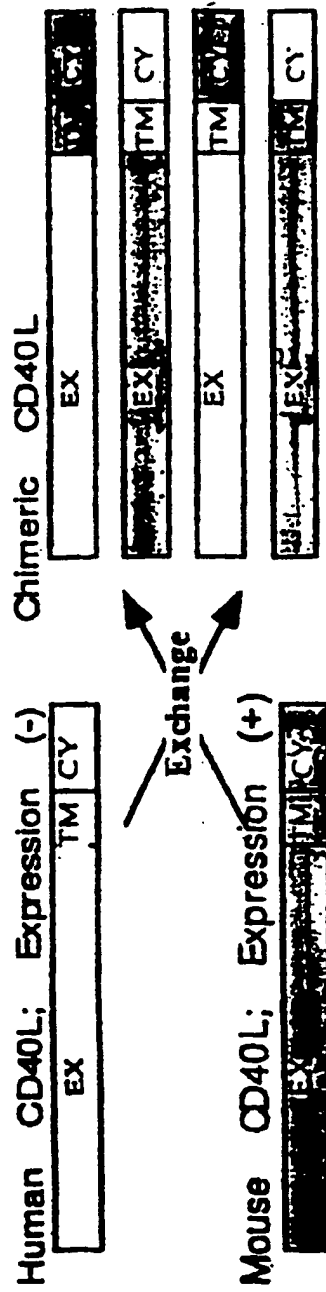


Figure 2

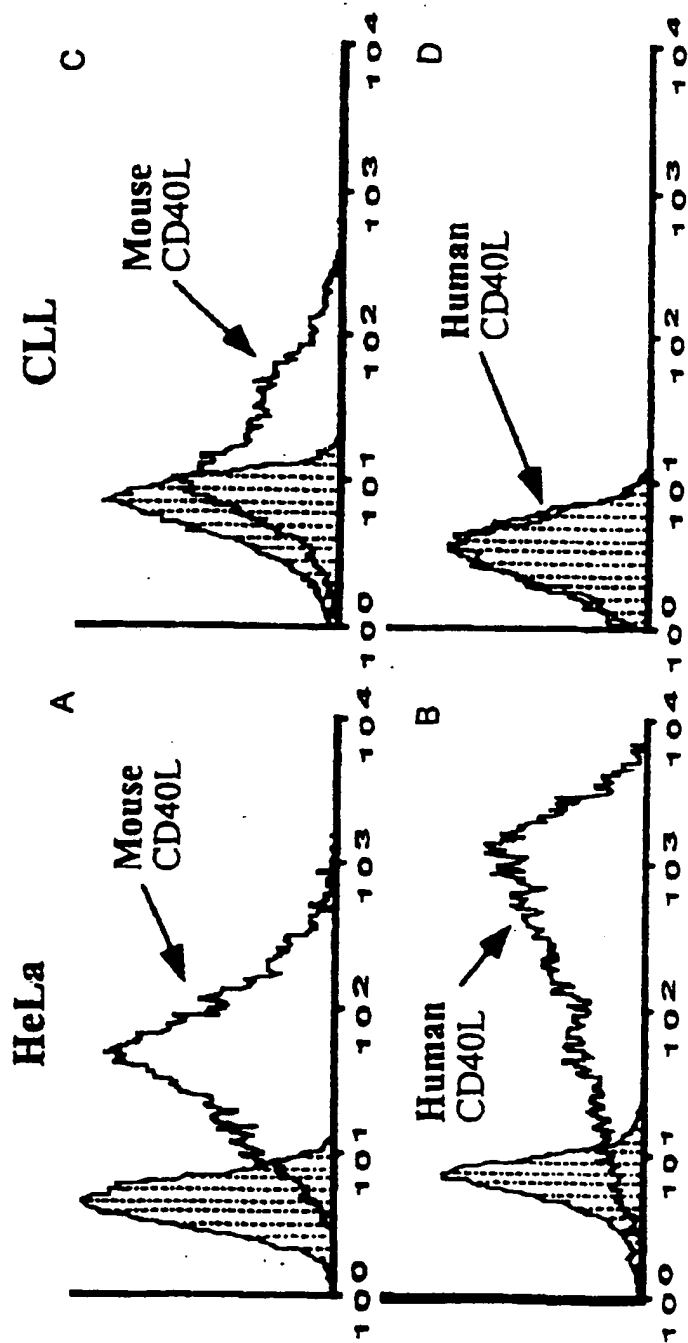


Figure 3

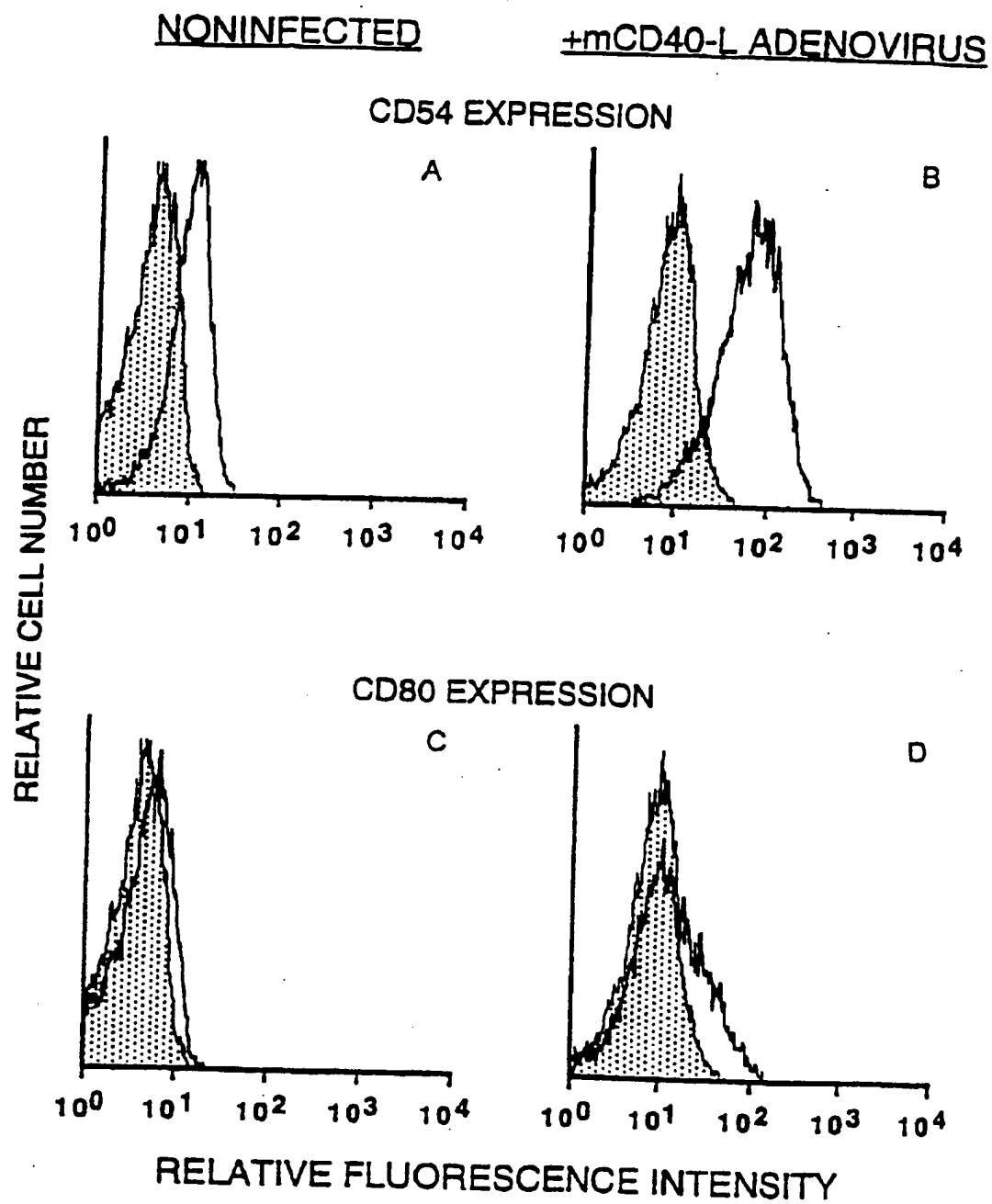


Figure 4

5/30

Allogenic T cell response to CLL cells transfected with adeno-mCD40L

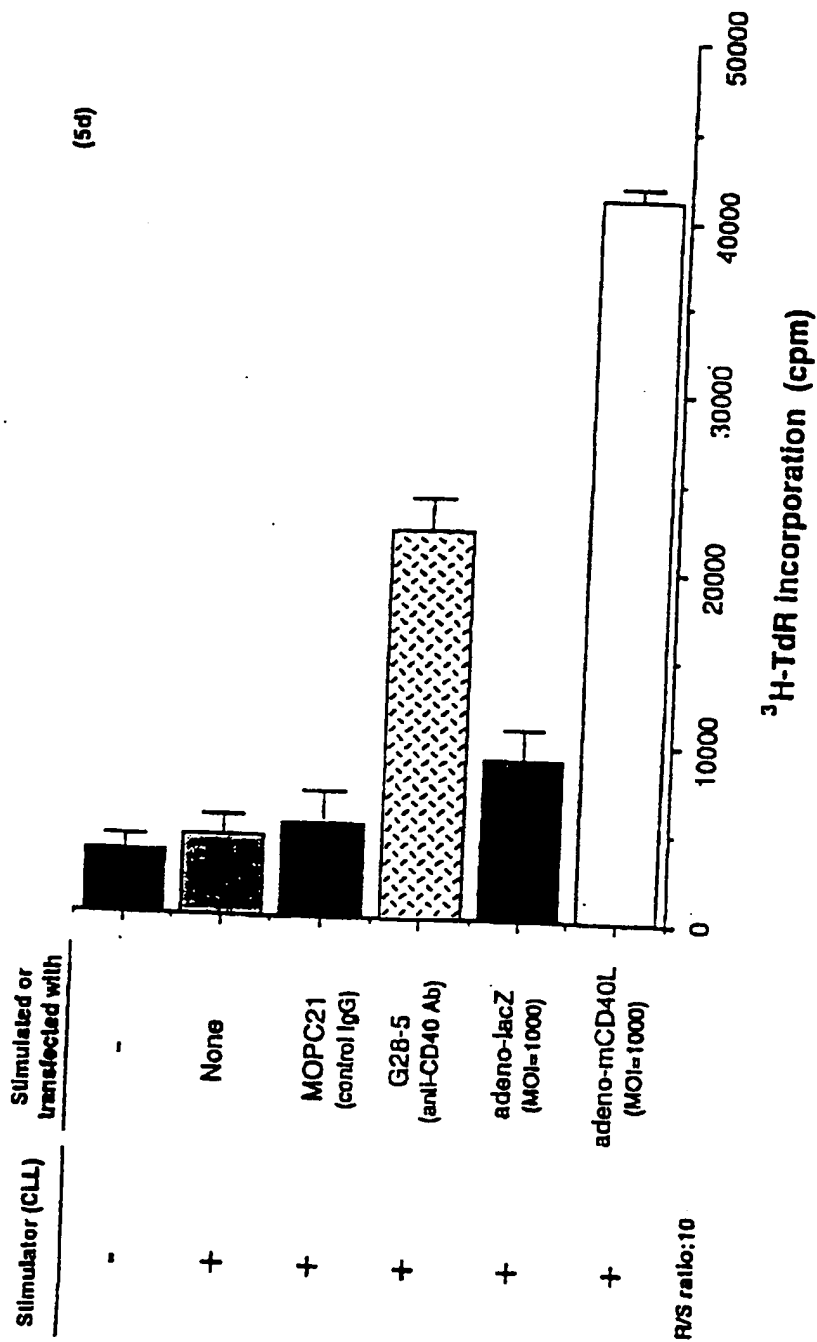


Figure 5

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**Production of IFN γ by allogenic T lymphocytes
stimulated with CLL B cells**

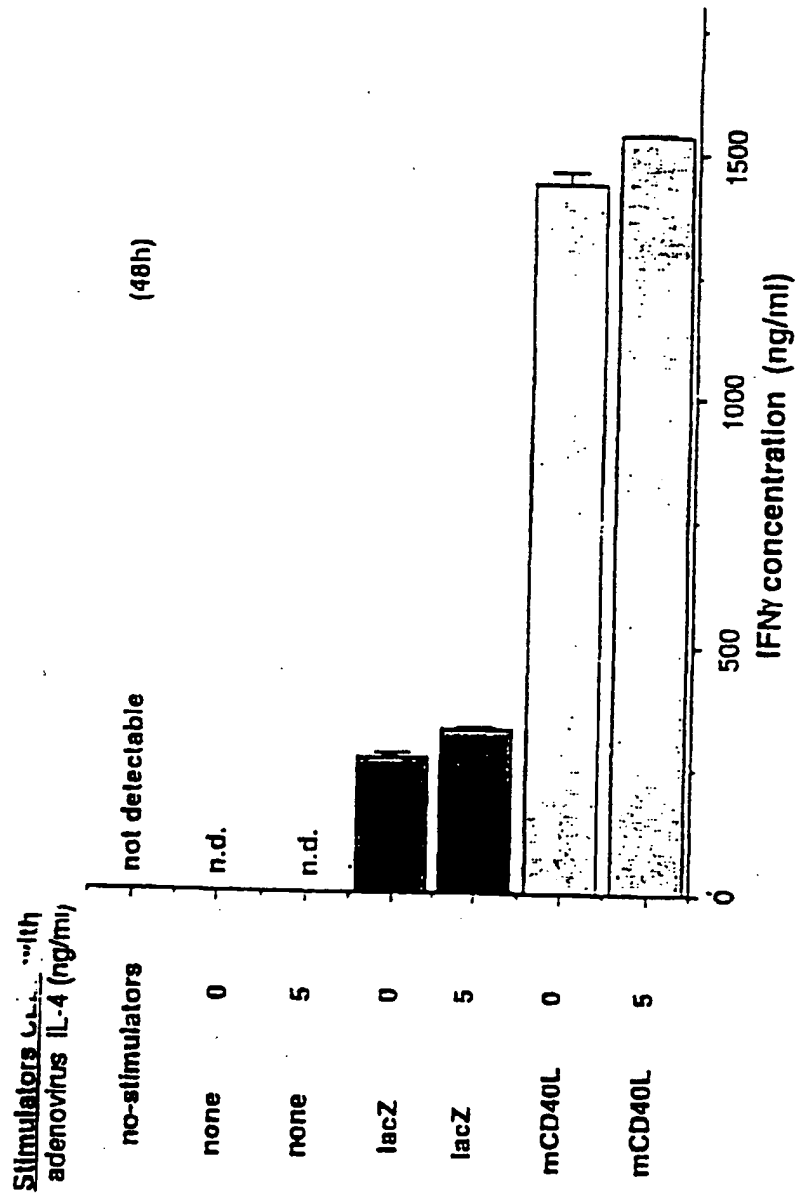


Figure 6

7/30

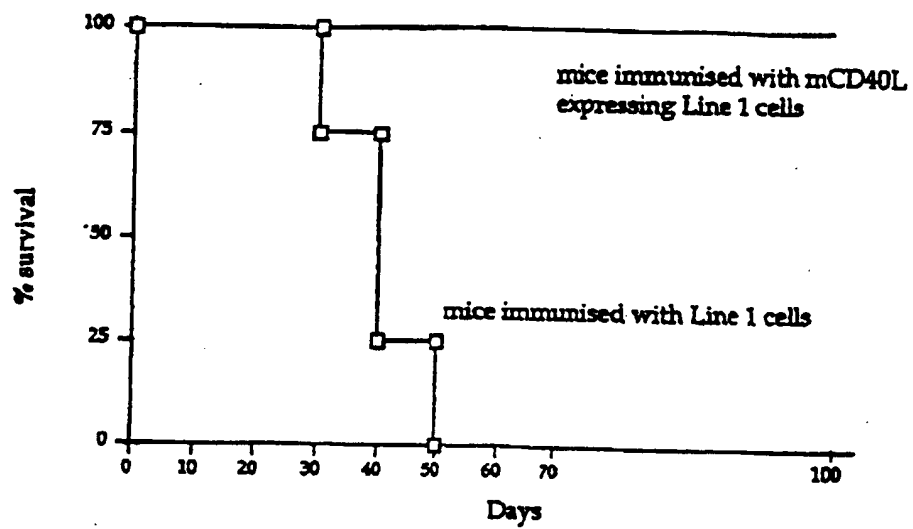


Figure 7

8/30

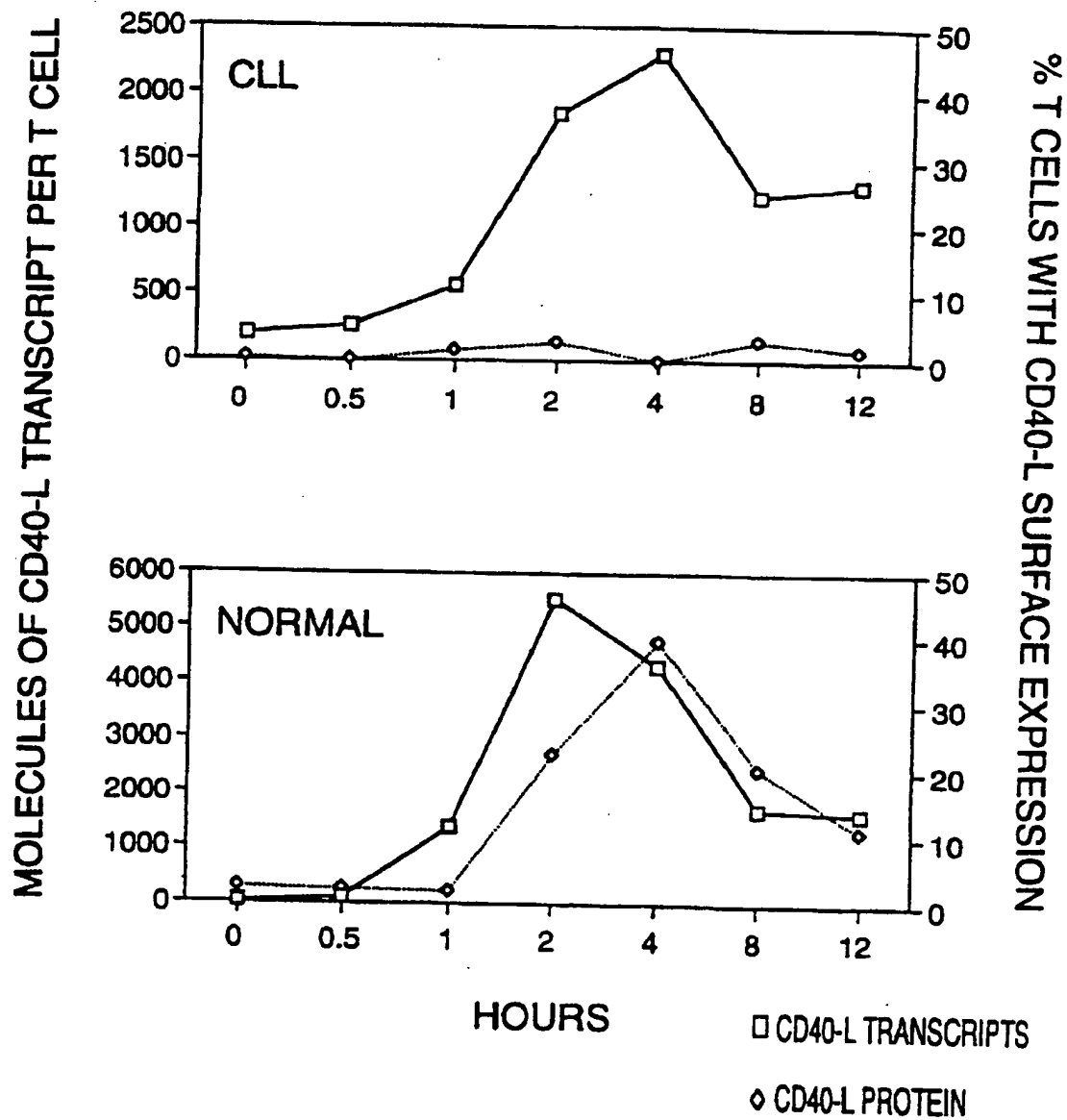


Figure 8

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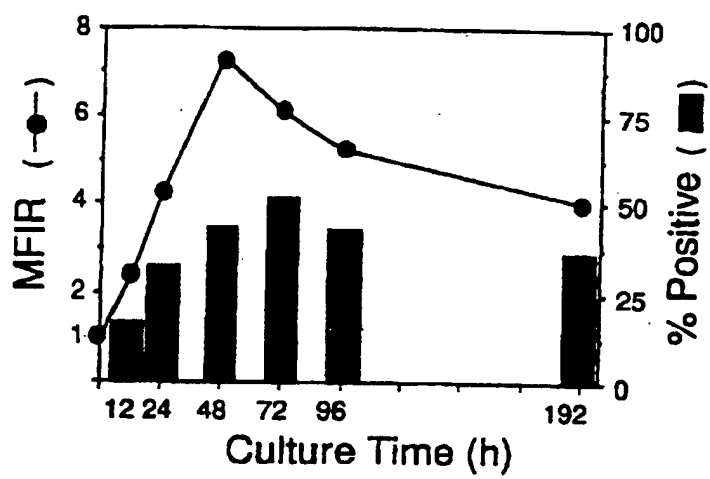


Figure 9

10/30

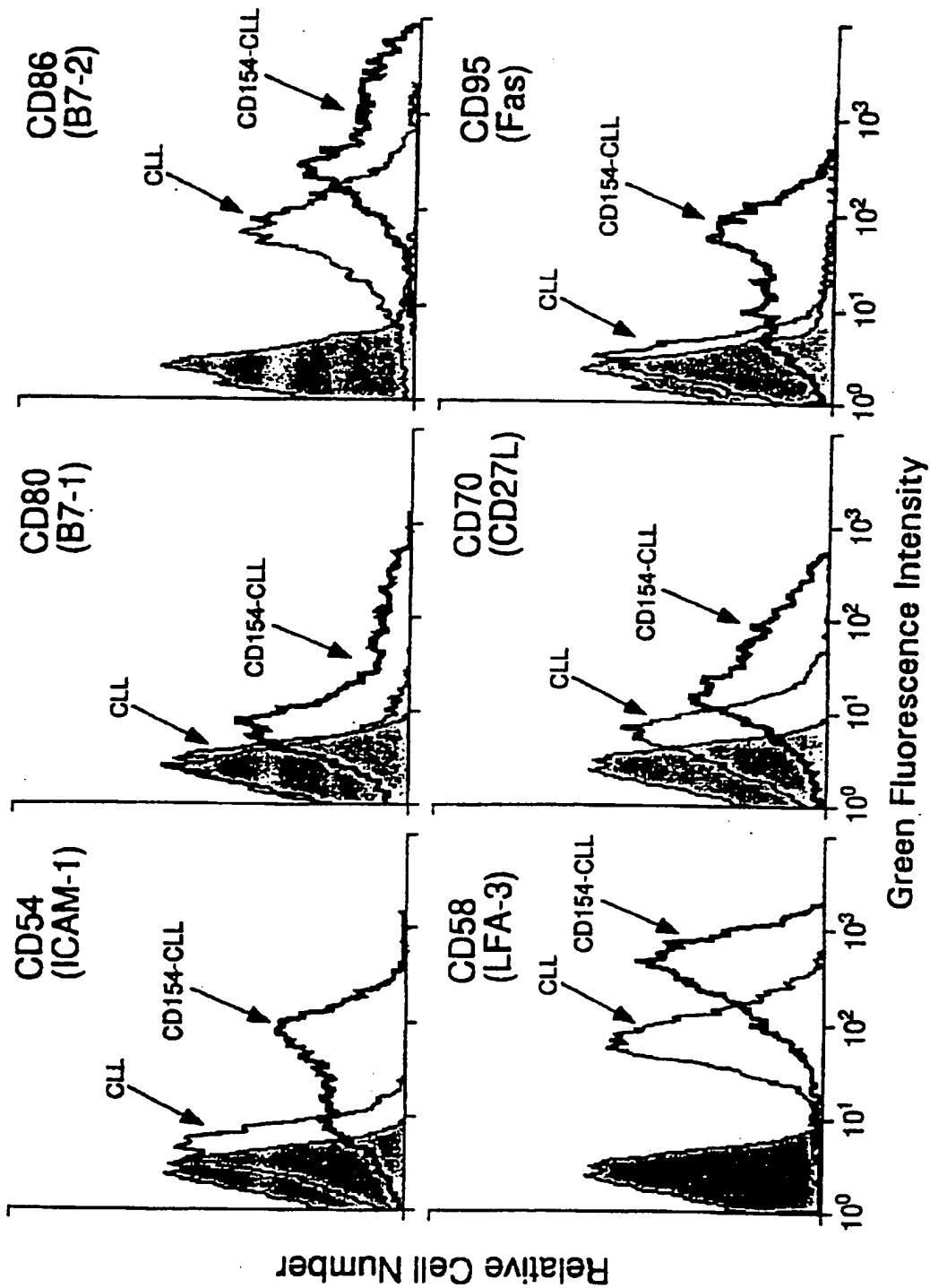


Figure 10

11/30

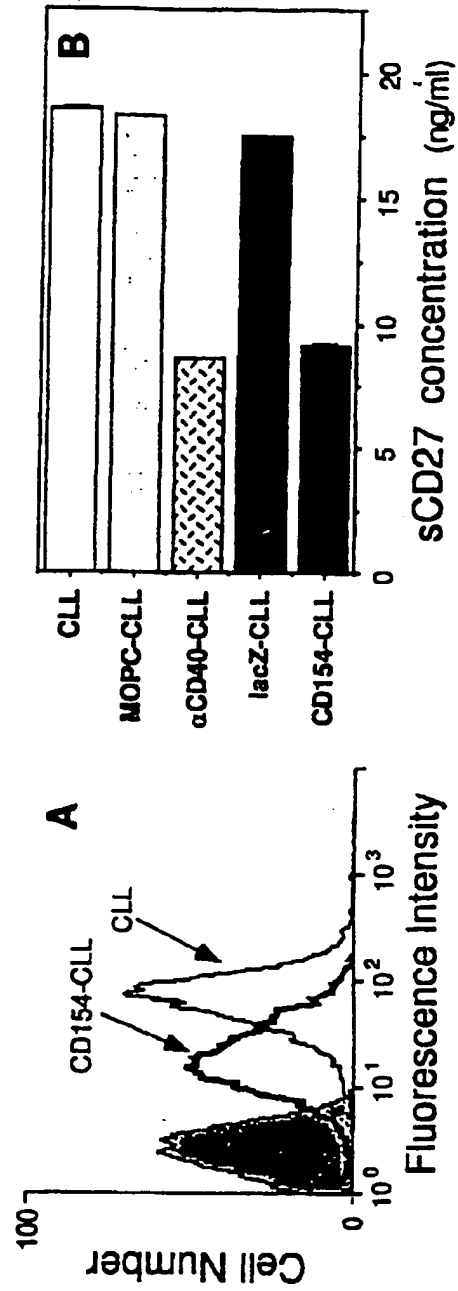


Figure 11

12/30

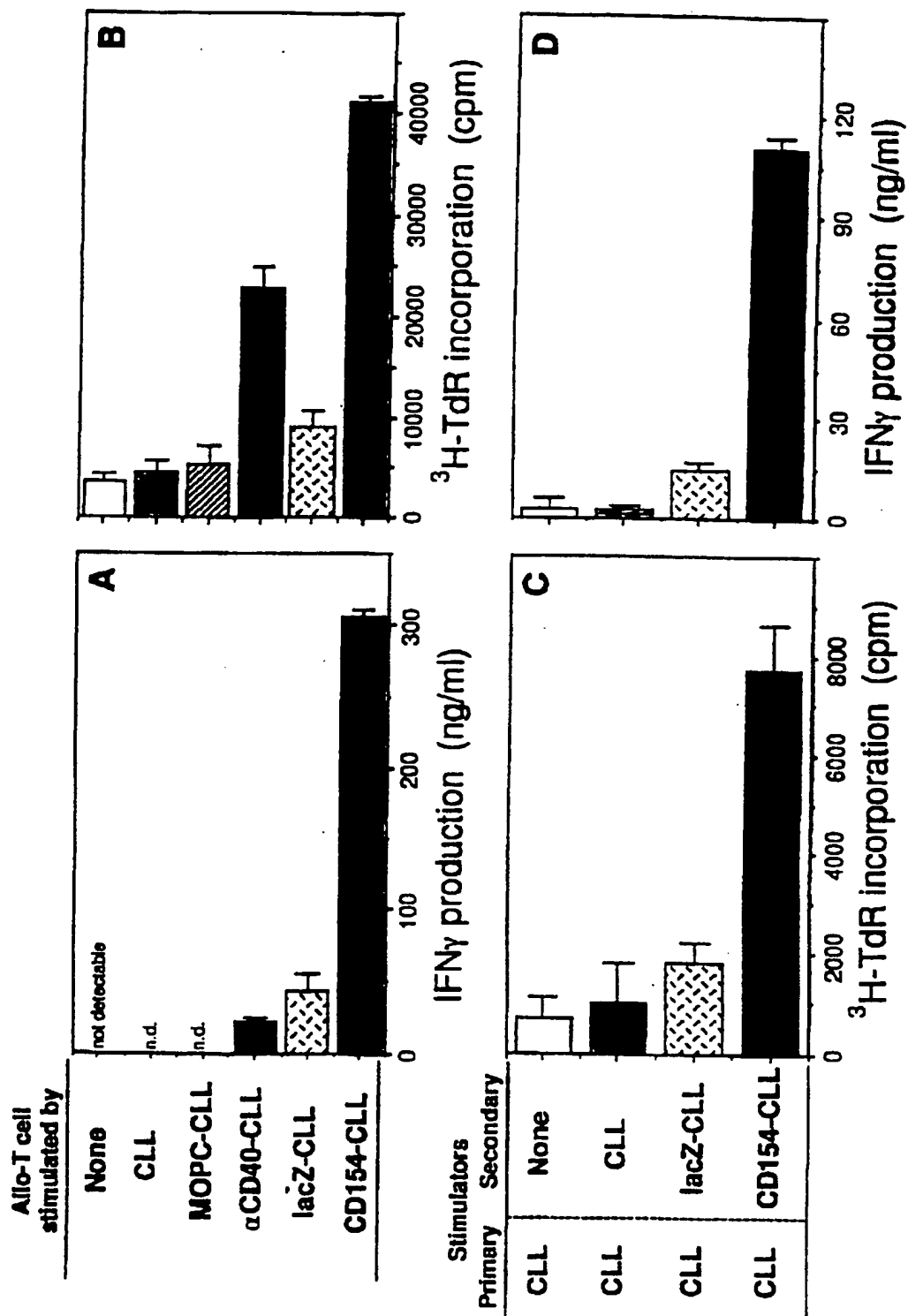


Figure 12

13/30

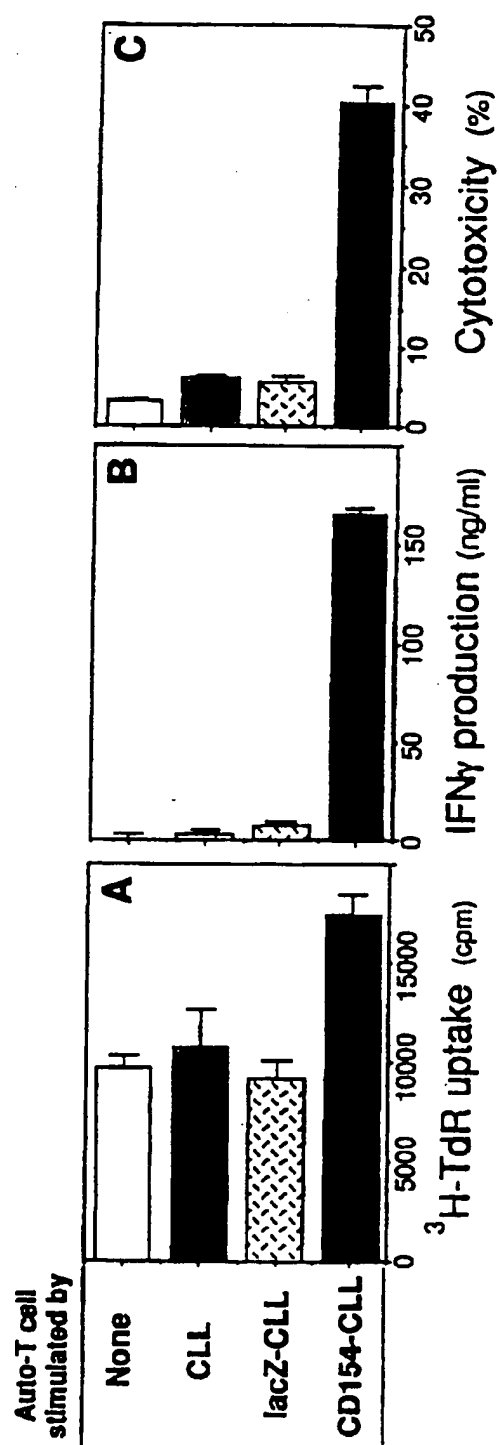


Figure 13

14/30

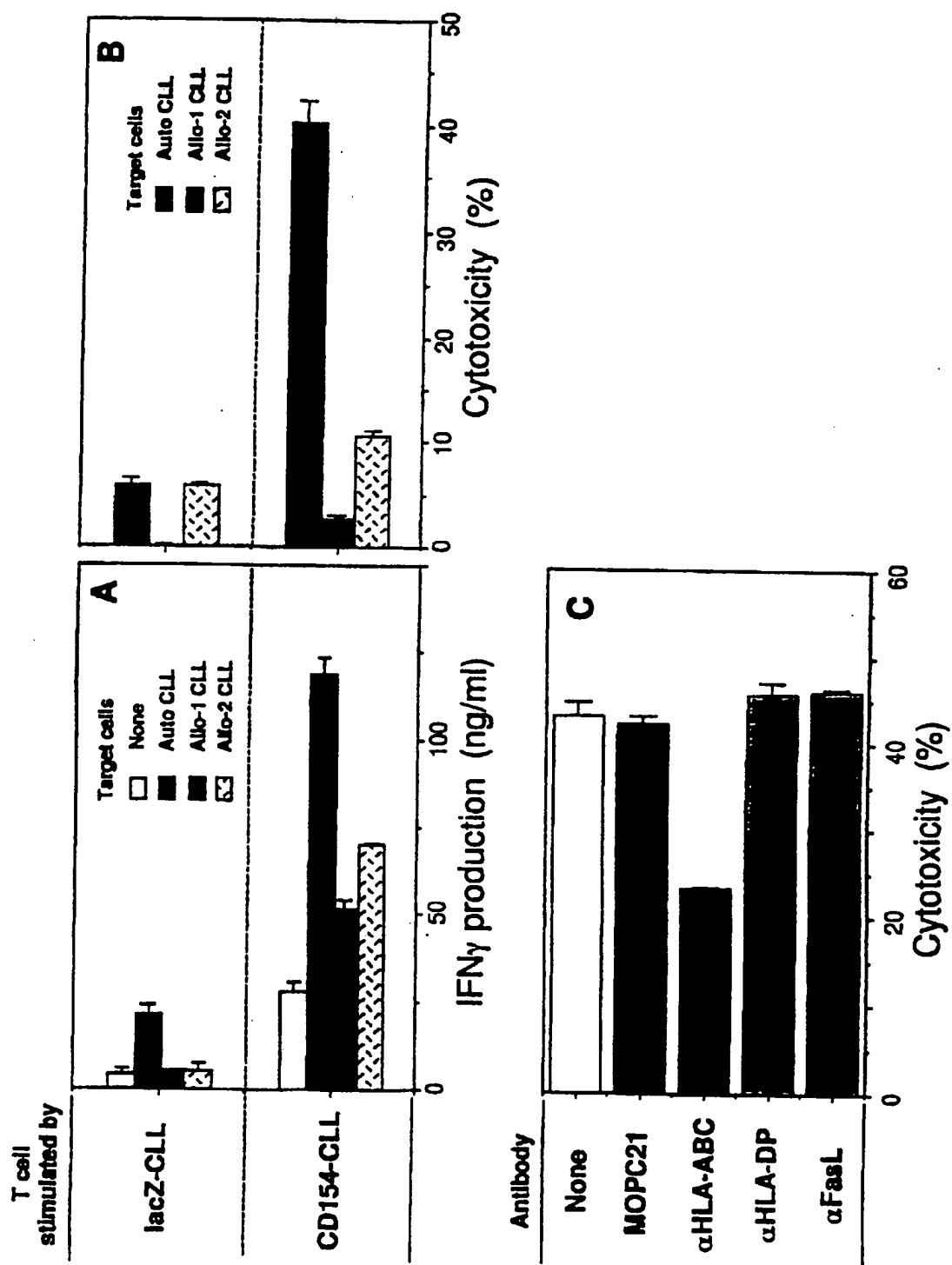


Figure 14

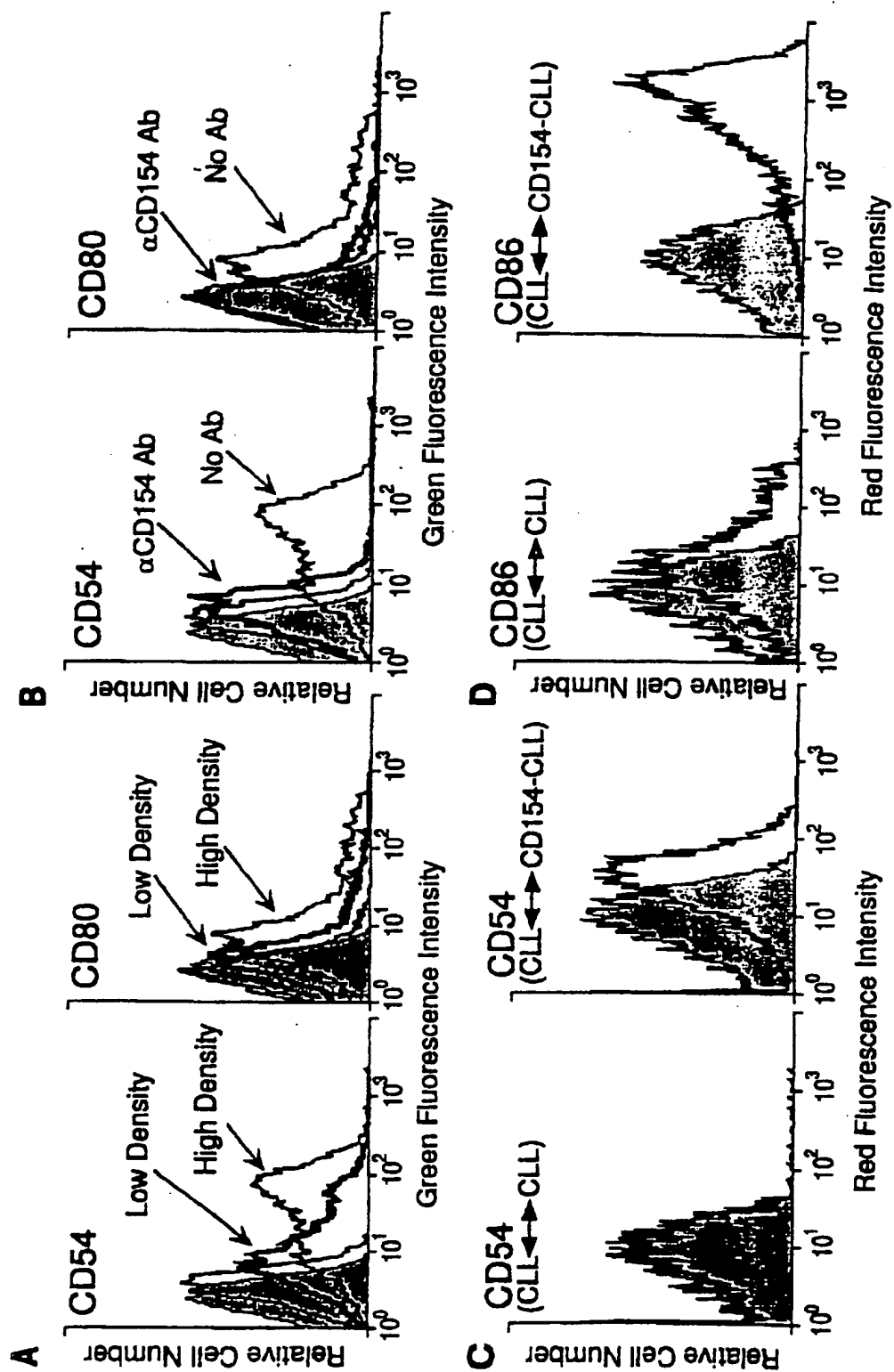


Figure 15

16/30

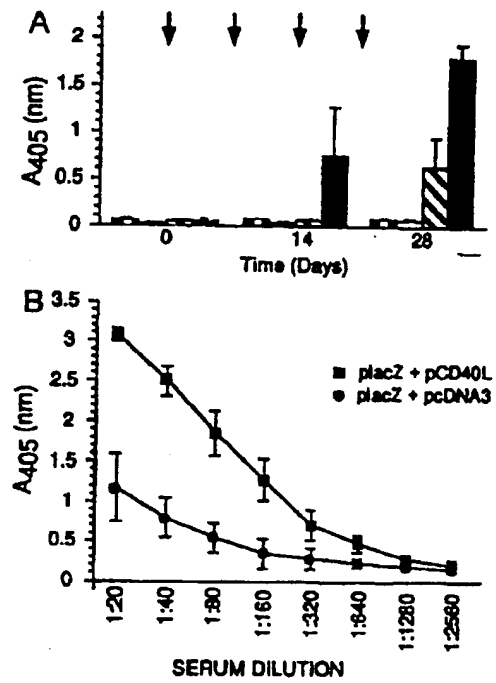


Figure 16

17/30

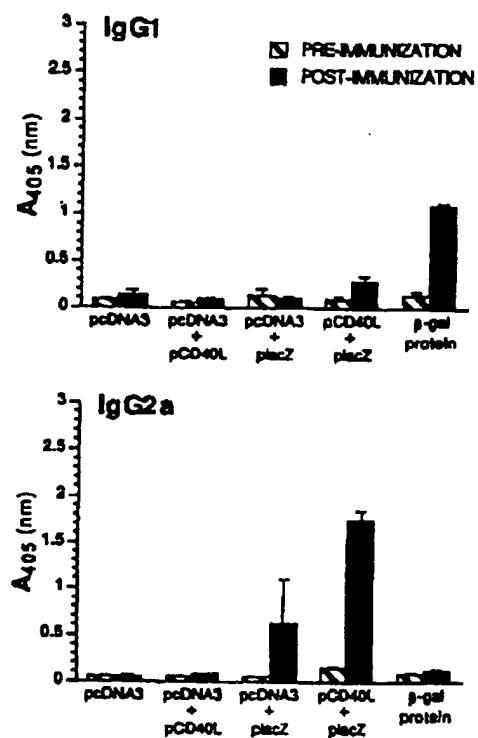


Figure 17

18/30

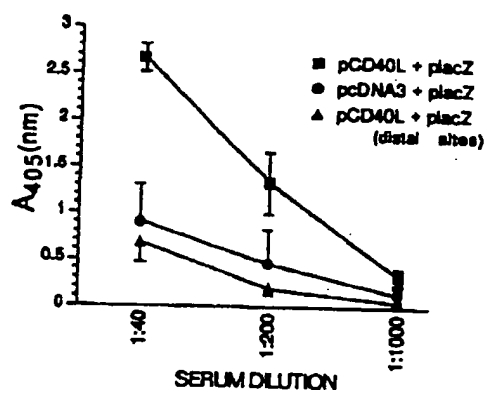


Figure 18

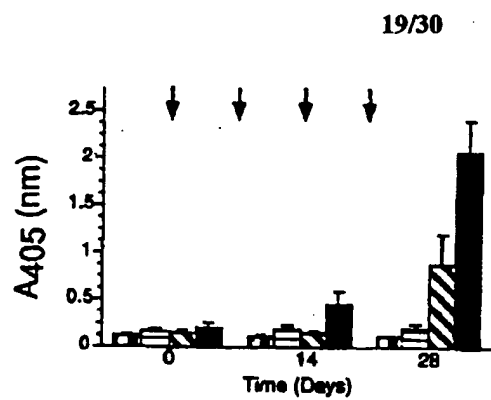


Figure 19

20/30

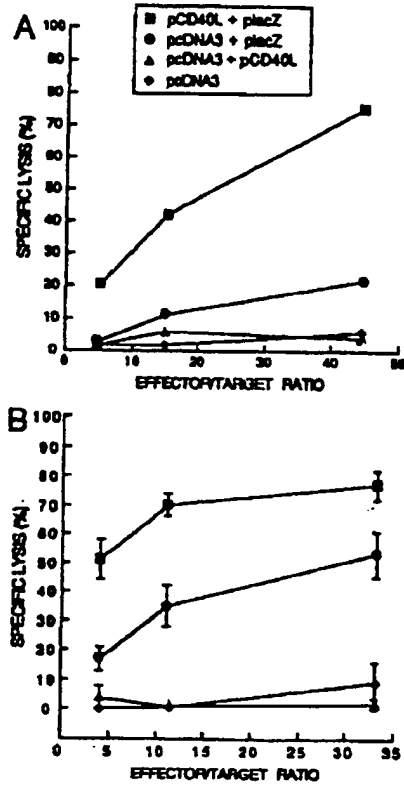
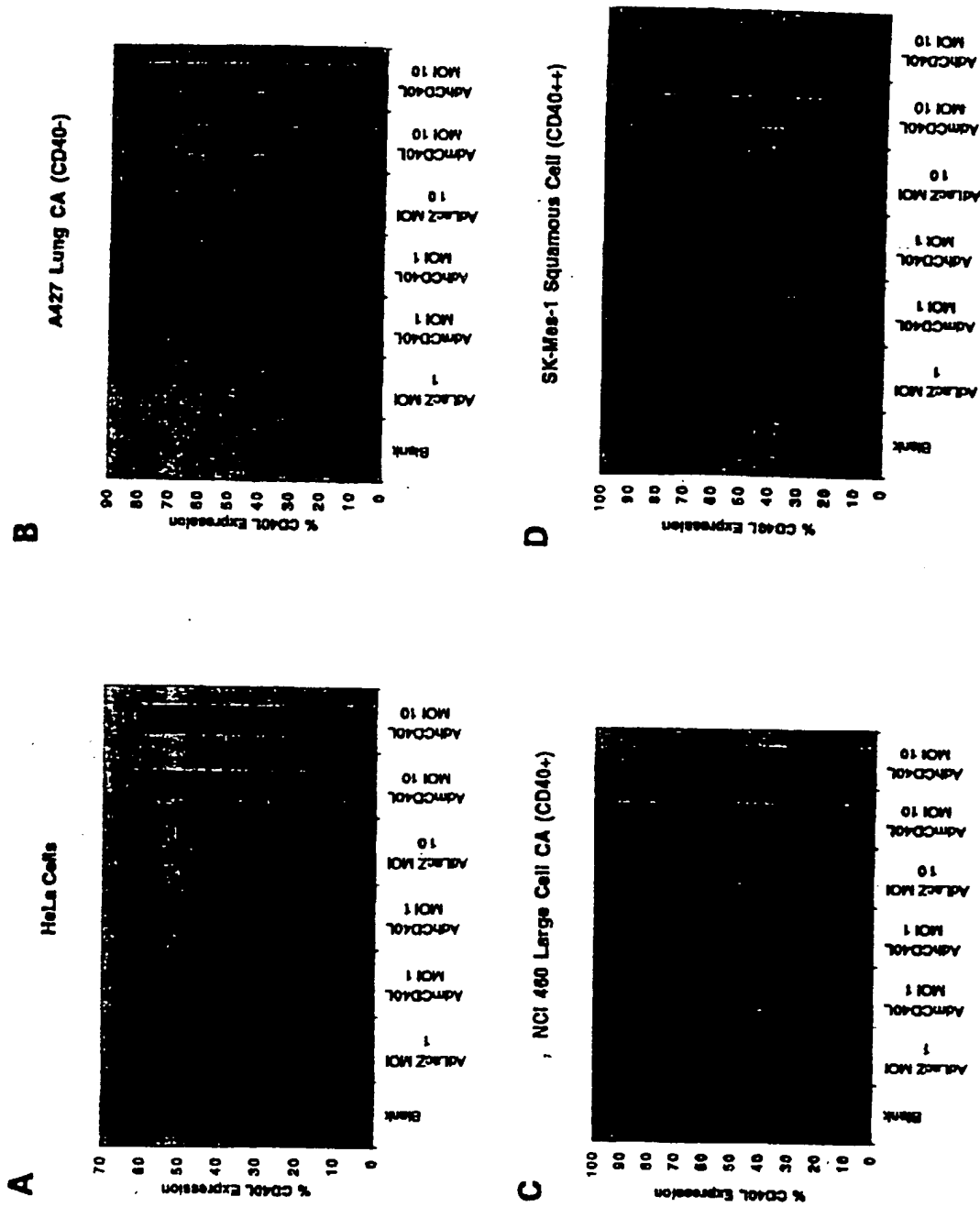


Figure 20



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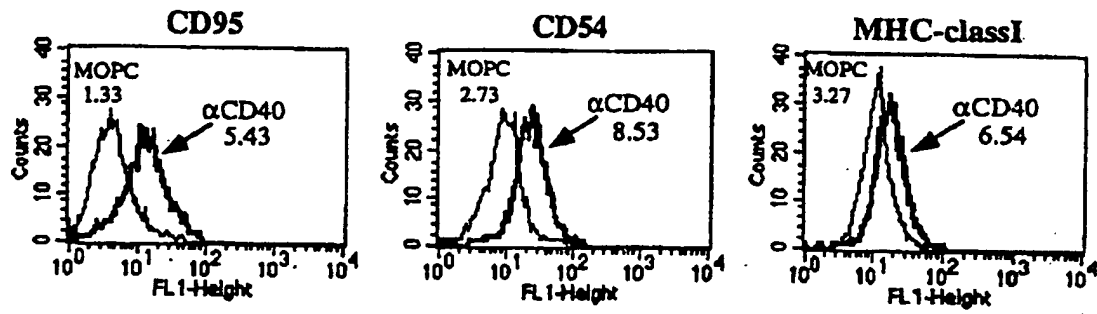
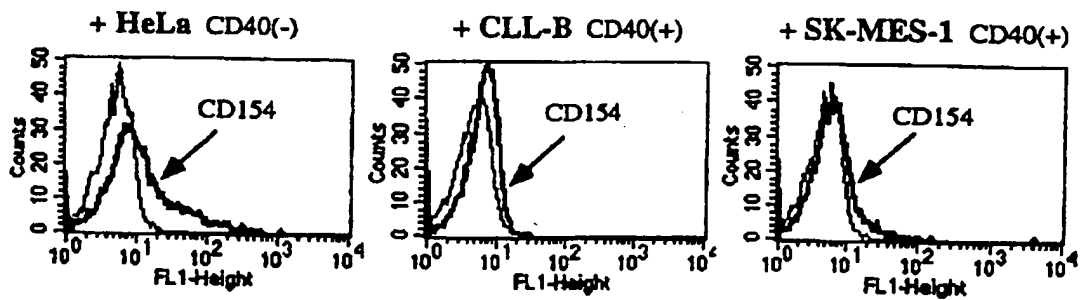
A**B**

Figure 22

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RA SYNOVIAL FLUID AND PLASMA INHIBITION OF FAS-LIGAND

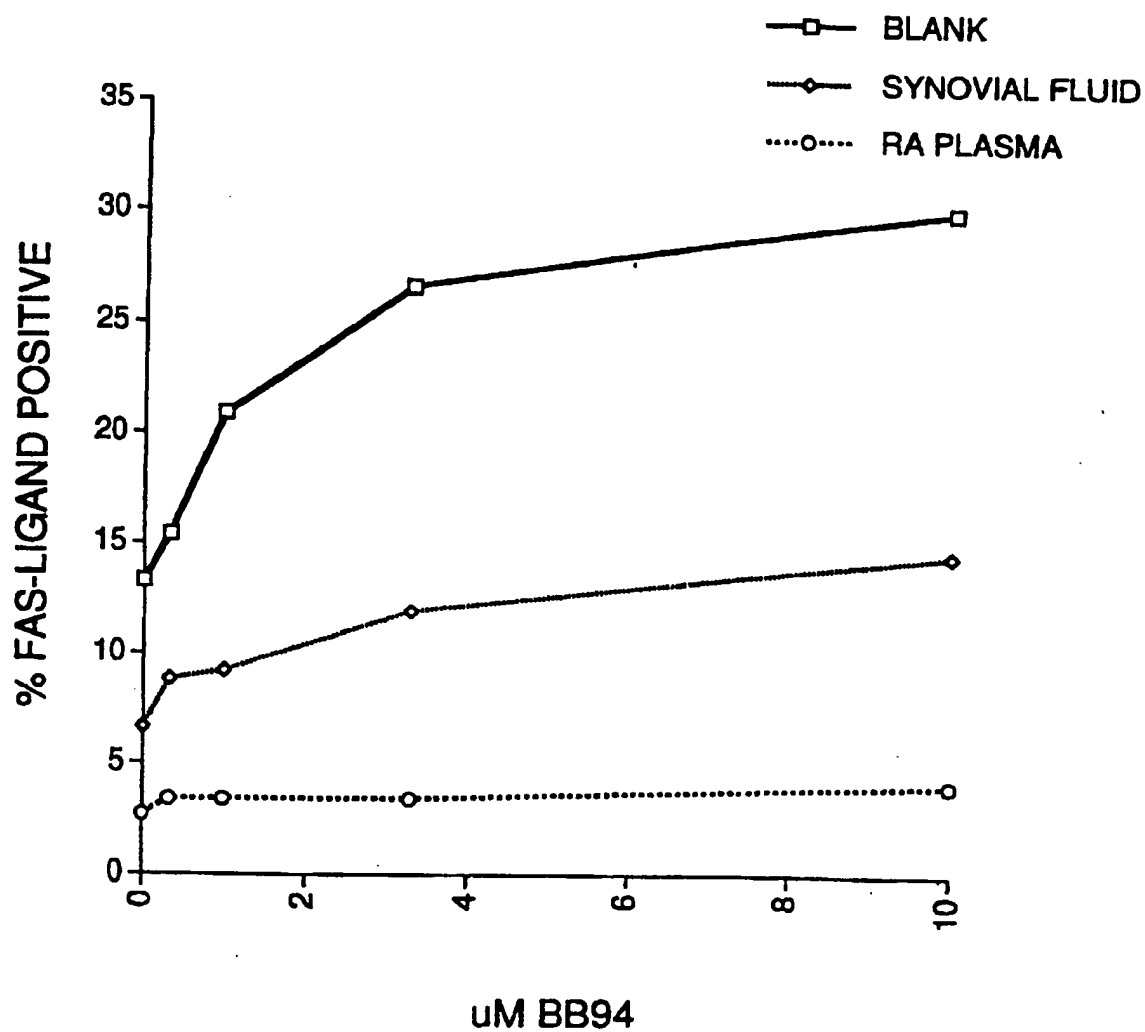


Figure 23

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Gene Therapy of Leukemia

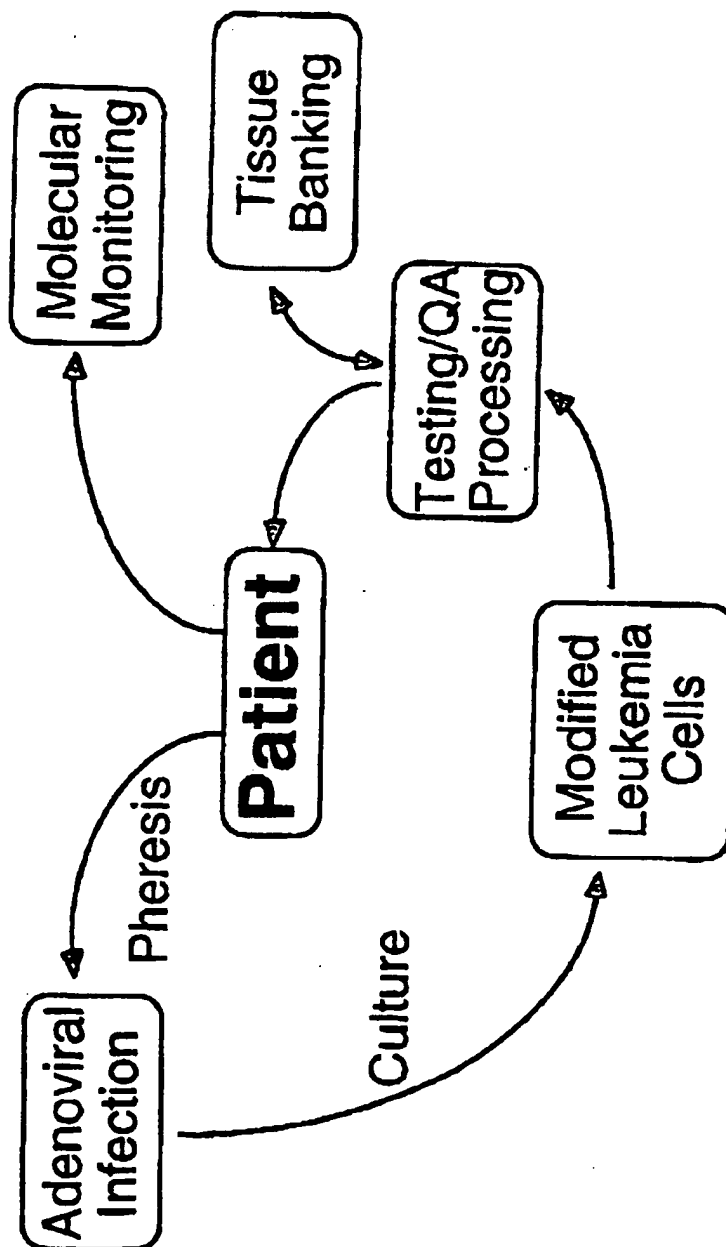


Figure 24

25/30

```
1  MQQPFNYFPYQIYWVDSSASSPWAPPGTVLPCPTSVPRRPGQRRPPPPPP 50
  |||||
1  MQQPFNYFPYQIYWVDSSASSPWAPPGTVLPCPTSEVPRRPGQRRPPPPPP 50
  |||||
51  PPPLPPPPPPPPPLPPLPPLPPLKRGNEISTGLCLLVHFFMVLVALVGLGLG 100
  |||||
51  PPPLPPPPPPPPPLPPLPPLPPLKRGNEISTGLCLLVHFFMVLVALVGLGLG 100
  |||||
101 MFQLFHLOKELAELESTSQMTASSLERQIGHPSPPPEKQELRKVAHLT 150
  |||||
101 MFQLFHLOKELAELESTSQMTASSLERQIGHPSPPPEKQELRKVAHLT 150
  |||||
151 GKSNSRSMPLWEDTYGIVLLSGVKYKRGGLVINETGLYFVYSKYVFRGQ 200
  |||||
151 GKSNSRSMPLWEDTYGIVLLSGVKYKRGGLVINETGLYFVYSKYVFRGQ 200
  |||||
201 SCNNLPLSHKVYRNNSKYPODLVMEGQNSYCTTGQNNARSSYLGAFFN 250
  |||||
201 SCNNLPLSHKVYRNNSKYPODLVMEGQNSYCTTGQNNARSSYLGAFFN 250
  |||||
251 LTSADHLYVNVSELSLVNFESQTTFGLYKL 281
  |||||
251 LTSADHLYVNVSELSLVNFESQTTFGLYKL 281
  |||||
```

Figure 25

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```
1  MQQFFNYYPQIYWVDSASSPWAPPOTVLPCTSVPRRPGQRRPPPPPP 50
   |||||||||||||||||||||||||||||||||||||||||||||||
1  MQQFFNYYPQIYWVDSASSPWAPPOTVLPCTSVPRRPGQRRPPPPPP 50
   |||||||||||||||||||||||||||||||||||||||||||||||

51  PPFLPPPPPPPPFLPFLPFLPKKGGNHSTGLCLLVMTFNLVALVGLGLG 100
   |||||||||||||||||||||||||||||||||||||||||||||||
51  PPFLPPPPPPPPFLPFLPFLPKKGGNHSTGLCLLVMTFNLVALVGLGLG 100
   |||||||||||||||||||||||||||||||||||||||||||||||

101 MPQLFHLQKELAELESTSOMHTASSLEKIGHPSPPPEKCELKVAHIT 150
    |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
101 MPQLFR.....FAQAIGHPSPPPEKCELKVAHIT 130
    |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||

151 GKSNSRSMPLEWEDTYGIVLLSGVKYKGGGLVINETGLYFVYSKVYFRQ 200
   |||||||||||||||||||||||||||||||||||||||||||||||
131 GKSNSRSMPLEWEDTYGIVLLSGVKYKGGGLVINETGLYFVYSKVYFRQ 180
   |||||||||||||||||||||||||||||||||||||||||||||||

201 SCNNLFLSHKVYMRNSKTPQDLVMEGKMSYCTTGQMAASSTLGAVFN 250
   |||||||||||||||||||||||||||||||||||||||||||||||
181 SCNNLFLSHKVYMRNSKTPQDLVMEGKMSYCTTGQMAASSTLGAVFN 230
   |||||||||||||||||||||||||||||||||||||||||||||||

251 LTSADHLYVNVSELSLVNFEESTFFGLYKL 281
   |||||||||||||||||||||||||||||||
231 LTSADHLYVNVSELSLVNFEESTFFGLYKL 261
   |||||||||||||||||||||||||||||||
```

Figure 26

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```
1  MQQPFNYFPQIYWVDSSASSFWAPPQTIVLPCPTSVPRAPGQRRPPPPPP 50
1  MQQPFNYFPQIYWVDSSASSFWAPPQTIVLPCPTSVPRAPGQRRPPPPPP 50
51  PPPLPPPPPPPLPPLPLPPLNKRGNHSTGLCLLVNFMVLVALVGLGLG 100
51  PPPLPPPPPPPLPPLPLPPLNKRGNHSTGLCLLVNFMVLVALVGLGLG 100
101 MFQLFHLQCLAKLRSTSCMTASSLEKQOIGHPSPPPFEGKELRKVAH/T 150
101 MFQLF.....MPFEGSCSVRRRPYGCYLRIGHPSPPFEKKELRKVAH/T 145
151 GKSNSRSMPLNEDTYGIVLLSGVKYKGGGLVINETGLYFVYSKVYFRQQ 200
146 GKSNSRSMPLNEDTYGIVLLSGVKYKGGGLVINETGLYFVYSKVYFRQQ 195
201 SCNLPLSHKVMRNSKYPQDLVMEGQMSYCTTQGMARSSYLGAFTN 250
196 SCNLPLSHKVMRNSKYPQDLVMEGQMSYCTTQGMARSSYLGAFTN 245
251 LFSADHLYVNVSELSLVNFESQTFPGLYKL 281
246 LFSADHLYVNVSELSLVNFESQTFPGLYKL 276
```

Figure 27

Matrix Metalloproteinase Cleavage Sites

Cleavage
↓

P ₄	P ₃	P ₂	P ₁	P' ₁	P' ₂	P' ₃	P' ₄
Collagenases							
MMP-1							
P ₄	P ₃	P ₂	P ₁	P' ₁	P' ₂	P' ₃	P' ₄
Ala	Pro	Leu	Gly	Met	Arg	Met/Ala	Arg
Gly/Leu	Leu	Met/Tyr	His	Leu	Leu	Gly	Lys
Met	Ala	Val/Gly	Glu	Ile	Phe	Val	Gln
Glu	Asp	Ile	Tyr	Gln	Trp	Ser	Ile
Pro	Ser	Gln/Arg	Ala	Pro	Glu	Glu	Gly
Tyr	Glu	Asp	Phe	Phe	Ala	Phe	S r
Ile	Gly	Glu	Gln	Ala	Val/Gly	Arg	Glu
Thr	Arg	Ala	Asn	Tyr/Val	Ser	Pro	Ala
Arg				[not K,E,W]	Asn		
MMP-8							
P ₄	P ₃	P ₂	P ₁	P' ₁	P' ₂	P' ₃	P' ₄
Ala	Pro	Leu	Glu	Tyr	Ala	Gly	Arg
Gly/Leu	Leu	Gln	Gly/His	Ile	Leu	Met	Gln
Met			Ala	Leu	Trp	Ala	
Glu				Val			
Pro			Ala	Phe			
Tyr/Ile/Thr/Arg							
(otherwise same as MMP-1)							

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Figure 28A

Gelatinases

Gelatinase A

P₄ Gly Ile Pro Arg Leu

P₂
Arg
Gln
Leu
Ala
Lys
Ile
His

P₁ Gly Asn Ala His Leu Tyr

P'1
Leu
Ile/Phe
Val/Met
Ala
Glu
Gln/Asn
Ser

P₂
Ala/Leu
Phe/Trp
Gly
Arg/Gln
His
Val

P₃
Gly/Ala
Leu
Ser
Pro

P¹ Gln Arg His Pro

MMP-9
P₄
Gln/Arg

Gelatinase B

P₂
Arg
Gln
Leu

P¹Gly

P¹₁
Leu
Ile/Phe
Val/Met
Ala

**P'2
Glu
A1a/Leu/Phe**

P',
Ala
Leu
Ser
Gly

P. 4

Stromelysins

MMP-3 Stromelysin 1

P₄
Asp
Gly
Gln/Arg
Leu
Ile
Glu/Val
Leu
Lys
Gly/Asp
Arg
Ser/Lys/Ile
Pro/Met
Ala/Phenyl

P₂
Phe
Leu/Met

P₁ Glu Ala

P',
Leu
Phe

P'2
Arg
Leu/Phe

P'3
Ala
Arg/Met

Thir

Tyr
Pro/Gly/Glu

Gln/Ph
Asn

Trp/Tyr

Val	Trp
-----	-----

Gly

Pro

Leu
Lys
Gly/Asp
Arg
Ser/Lys/Phe
Pro/Met
Ala/Phe/Gln

Ile
Ala
Ser

His
Gly

Lew/Pro

Lys/Tyr/Arg

Val
Met
Glu

Gln
His/Met
Glu/Ser/Thr

Ser/Asn
Glu/Thr

Ala

Pro/Met
Ala/Phe/Gln
Ser/Gly

Lys/Tyr/Arg

Figure 28B

